Implementation and on-going improvement of an obstetric intra-operative cell salvage service

by

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DECLARATION

Whilst registered as a candidate for the above degree, I have not been registered for any other research award. The results and conclusions embodied in this thesis are the work of the named candidate and have not been submitted for any other academic award.

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I must also pay tribute to fellow researchers, Mr John Faulds and Dr Catherine Ralph who without their encouragement I would not be where I am now.

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ABSTRACT

Obstetric haemorrhage is still a leading cause of mortality and morbidity in what is essentially a young healthy population. Treatment for haemorrhage, outside surgical techniques, was replacement of blood loss with allogeneic (donor) blood. Although blood transfusion is a life-saving treatment, it is a limited resource with shortages, is increasingly expensive and is not without risk. Blood from the UK is one of the safest in the world, but there is still a risk of bacterial and viral transmission, as well as concerns with the quality. As such, alternatives need to be identified.

One such alternative is the process of intra-operative cell salvage. For many years, and still ongoing to some degree, obstetric cell salvage has been contraindicated, essentially based on two concerns: amniotic fluid embolus and red cell alloimmunisation.

The focus of my research has been the implementation of an intra-operative cell salvage service into our hospital, addressing the concerns and risks with it, and investigating the quality of the cell salvaged blood.

Following the research work undertaken, intra-operative cell salvage is now established in all caesarean sections 24 hours a day, seven days a week within our hospital; very few centres offer this. It is an essential component of our obstetric patient management strategy.

PREFACE

There are significant concerns with the safety and supply of allogeneic blood, and there is a requirement for alternatives to be sought. This thesis is divided into sections that explore these concerns with transfusion, the alternatives, the role of intra-operative cell salvage in obstetrics and the impact on our hospital transfusion rates, the quality of cell salvage blood and the future aspects.

The thesis provides a narrative that combines nine published peer reviewed articles that I have authored or co-authored over a ten-year period. In the appendices, these articles are included in their full text format, alongside my contribution within each article and the number of citations.

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ABBREVIATIONS

2,3-DPG	2,3-diphosphoclycerate
AAGBI	Association of Anaesthetists of Great Britain and Ireland
AF	Amniotic fluid
AFP	Alpha-fetoprotein
AFE	Amniotic fluid embolus
C/S	Caesarean section
DIC	Disseminated intravascular coagulation
EBL	Estimated blood loss
FMH	Fetal-maternal haemorrhage
HDFN	Haemolytic disease of the fetus and newborn
ICS	Intra-operative cell salvage
LDF	Leucodepletion filter
LDH	Lactate dehydrogenase
NHS	National Health Service
NHSBT	National Health Service Blood and Transplant
NICE	National Institute for Health and Care Excellence
PPH	Post-partum haemorrhage
QC	Quality control
RCH	Royal Cornwall Hospital – part of the Royal Cornwall NHS Hospitals Trust
RCOG	Royal College of Obstetricians and Gynaecologists
SHOT	Serious Hazards of Transfusion
UKCSAG	UK Cell Salvage Action Group

CHAPTER 1 – INTRODUCTION

Obstetric haemorrhage is still a leading cause of mortality and morbidity in what is essentially a young healthy population. The most recent report (published 15th June 2018) on maternal health 'Saving Lives, Improving Mothers' Care' MBRRACE-UK (Mothers and Babies: Reducing Risk through Audits and Confidential Enquiries across the UK) covering the period 2013-2015 showed that 22 women died from haemorrhage, equating to a mortality rate of 0.88 (Knight et al., 2017). Despite advances in medicine, there has not be an improvement in the number of deaths from haemorrhage. In fact, there has been no improvement since 1994, with this period 2013-15 seeing the highest mortality rate. The World Health Organisation reported that 25% of maternal deaths worldwide are due to severe haemorrhage (World Health Organization, The World Health Report, 2005).

Treatment for haemorrhage, outside surgical techniques, was replacement of blood loss with allogeneic (donor) blood. Although blood transfusion is a life-saving treatment, it is a limited resource with shortages, is increasingly expensive and is not without risk. Alternatives are now being sourced.

In the UK, the demand for donor blood is decreasing, however, the supply rate is also decreasing, but at a quicker rate. There is also an increased demand for certain blood groups which is outstripping supply, putting significant pressures on the National Health Service Blood and Transplant (NHSBT) (NHS Blood and Transplant Annual Review of Key Risks, 2017). For example, there is an increased demand for "universal" group O RhD negative blood. Approximately 7% of the population are O RhD negative, yet the demand from hospitals is approximately 13% (NHS Blood and Transplant

Annual Reports and Accounts, 2018). Only 4% of the eligible population donate, and there is a continuous need to replace donors who can no longer donate (Joint UK Blood Transfusion and Tissue Transplantation Services, 2014). The impact on the supply chain is continuously assessed by NHSBT and is flagged at their annual board meetings of risk review (NHS Blood and Transplant Annual Review of Key Risks, 2017). The demand-supply issue is being addressed.

Blood in the UK is one of the safest in the world, but there is still a risk of bacterial and viral transmission such as human immunodeficiency disease, hepatitis A, B and C virus, human T-cell leukaemia virus, cytomegalovirus, Epstein-Barr virus, human parvovirus B19, West Nile virus, dengue virus and malaria, to name some (Mollison et al., 1997). The risk increases in countries with a low human development index (Marcucci et al., 2004).

Prion disease (variant Creutzfeldt-Jakob disease) is another concern with a confirmed risk of transmission from blood transfusion. To date there have been four reported cases of infection post transfusion in the UK. The UK blood services implemented changes to reduce this risk, however, there is still no screening test available. The initial outbreak of variant Creutzfeldt-Jakob disease seems to be over, but a further outbreak has not been entirely ruled out (Seed et al., 2018). In 2015 it was identified that hepatitis E virus can be transmitted through an allogeneic blood transfusion and hepatitis E virus negative components should be provided for certain populations. In 2017 it was recommended that NHSBT undertake universal screening of all units for hepatitis E virus (SaBTO, 2017). This additional screening of all blood added a

significant financial cost to the National Health Service (NHS), which is reflected in the increasing cost of a unit of blood.

There are also concerns with the quality of allogeneic blood, such as reduced oxygen carrying capacity (low 2,3-diphosphoglycerate (2,3-DPG) concentrations), reduced adenosine-triphosphate concentrations, increased leakage of potassium, increased red cell rigidity (which will affect the ability of the cells to pass through the circulation) and blood becoming increasing acidotic with storage. My unpublished work has shown potassium concentrations increase over time with storage, with concentrations of 24.8 mmol L^{-1} to 51 mmol L^{-1} after day 35. There is also a strong correlation between the age of allogeneic blood and the pH (*Pearson's r* correlation -0.727), with the pH falling from 7.15 in five-day old blood to 6.65 at 35 days age.

2,3-diphosphoclycerate concentrations have been shown to fall as early as the third to fifth day of storage and by day 14 levels are almost at zero (Mollison et al., 1997). Low concentrations are associated with poor survival, however, once transfused, 2,3-DPG concentrations return to 50 percent after seven hours and normal levels within two to three days (Tinmouth et al., 2006; Yoshida & Shevkoplyas, 2010). The pH of the allogeneic red cell product also plays a critical role with 2,3-DPG, with concentrations rapidly decreasing when the pH falls below 7.2 (Beutler et al., 1969; Mollison et al., 1997; Zimrin & Hess, 2009).

Alongside the concerns with the quality and risks of blood, having an allogeneic blood transfusion alone can increase length of stay rates within the hospital, by 1.3% per unit transfused (Baker et al., 2014), and the rate of post-operative infections

(allogeneic blood suppresses the recipients immune system), which all increases demand and costs for hospitals. There are also increased risks of morbidity and five-year mortality with allogeneic blood (Duffy & Neal, 1996; Vamvakas, 2002; Davis et al., 2003; Karkouti et al., 2004; Innerhofer et al., 2005; Koch et al., 2006). Women who delivered at the Royal Cornwall Hospital (RCH) who have had an allogeneic transfusion are more likely to have a higher estimated blood loss and infections treated post-operatively, when compared to those who have had no transfusions (Baker et al., 2014).

Other effects of allogeneic transfusion include febrile reactions, haemolytic reactions, transfusion-related acute lung injury, graft-versus-host disease and citrate toxicity (Mollison et al., 1997). There are also reports of transfusion circulatory overload, which is now a leading cause of mortality or morbidity. Exposing patients to many allogeneic units increases the risks of sensitisation and producing antibodies which may complicate further crossmatching, or in our population of interest, haemolytic disease of the fetus and newborn (HDFN).

In the UK, there is an independent haemovigilance reporting scheme (Serious Hazards of Transfusion (SHOT)) which collects and assesses reported adverse events and reactions from blood transfusions and/or those involved in blood transfusions such as the NHSBT, hospital laboratories and clinical areas. Each year a report covering the previous year is published, which makes startling reading. The most recently published report (12th July 2018) covering the year 2017, identified 21 deaths where a blood transfusion was implicated, of which 14 were classed as preventable. There were a further 112 cases of major morbidity where a blood transfusion was involved

(Bolton-Maggs et al., 2018). ABO incompatible red cell transfusions are on the NHS “never event” list, and despite there being a reduction over time (three in 2016 and one in 2017) they still re-occur. There were a further 606 ABO-incompatible red cell near miss events in 2016 and 2017. In 2017, there were also four ABO incompatible transfusion involving fresh frozen plasma and two involving platelets. Alongside ABO incompatible transfusions, there were 326 reported cases of reactions to a blood transfusion, febrile or haemolytic, all of which are not preventable (i.e. is a risk of a transfusion). There were also 789 cases of wrong blood in tube incidences (i.e. blood in a sample to be tested is not from that patient recorded) which resulted in a near miss wrong component transfusion. Where an incorrect blood component was transfused, 51.5% were due to laboratory error with the remaining 48.5% due to clinical errors, of which the majority were due to collection of blood.

What this summary has shown are that there are serious complications with allogeneic blood transfusions, and regarding variant Creutzfeldt-Jakob disease and hepatitis E virus, it has shown that we do not know what is ‘around the corner’ with regard to emerging pathogens and the impact on the blood supply. We should therefore be reducing these risks as much as possible, and there is now a drive to review alternatives to allogeneic transfusion.

In 2012 a Patient Blood Management initiative was created by the NHSBT and National Blood Transfusion Committee along with the Department of Health to look at alternatives and support hospitals to manage their blood use effectively, reduce allogeneic transfusion rates, and ensure that patients receive the best treatment and

that avoidable, inappropriate use of blood and blood components is reduced (Joint UK Blood Transfusion and Tissue Transplantation Services, 2014).

One such alternative is the process of cell salvage which will be the focus of this commentary.

CHAPTER 2 – CELL SALVAGE

Cell salvage, simply put, is a method where lost patient blood is collected, washed, filtered and then re-infused to the patient. This is known as an autologous blood transfusion.

Autologous transfusion was first postulated in 1874, when a doctor who attended a woman bleeding post-delivery noted that even though blood loss was stopped, the woman died with “several pounds of blood in a vessel and in the bed”. He speculated that if the blood had somehow been collected and re-infused, it could have saved the woman’s life (Highmore, 1874). There is documented evidence of its use in 1874 (Halstead, 1883), with a successful case report published in 1886 which showed its potential. Higher than expected blood loss was experienced post leg amputation. Blood was collected and injected back into the femoral vein. The patient made a good recovery with no shock or change in temperature. The patient was very weak and anaemic pre-operatively and due to the haemorrhage experienced, it was concluded that if the blood was not given back, the patient was very unlikely to have survived and that the patient ultimately gained from having the re-infusion (Miller, 1886).

Since the late 1800’s to 1940’s there are documented cases of its use in ruptured ectopic, splenectomy, haemothorax, perforating abdominal injuries and neurosurgical procedures (Thies, 1914; Lockwood, 1917; Watson & Watson, 1936). Interest in autologous transfusion increased up to the Second World War when blood supply was not an issue due to increased donor numbers (Konig & Waters, 2012; Sikorski et al., 2017). Interest increased again post Korean and Vietnam wars, with the first commercially available autotransfusion device being introduced in 1968 (Klebanoff,

1970). In 1974, Haemonetics® Corporation launched the ‘Cell Saver’ which is still going strongly today, albeit with many major improvements over the years.

There are two forms of cell salvage: post-operative cell salvage (used mainly in orthopaedics) and intraoperative cell salvage (ICS), with the latter being the focus here.

There are different models of cell salvage machines available, but specifically to our hospital is the model that uses the “Latham” style bowl and process. Blood lost is collected via a dual lumen suction tube which mixes with an anticoagulant (either heparin or acid citrate-dextrose anticoagulant) into a collection reservoir through a filter to remove large clots and debris. Filters are usually of the size 40-150 µm. Once a sufficient volume has been collected, blood is pumped into the Latham centrifugal bowl where it undergoes centrifugal forces. Denser red cells move to the outside of the bowl, whilst the less dense plasma, contaminants, free haemoglobin, platelets and clotting factors move towards the centre of the bowl and exit to waste. Once sufficient red cells remain in the bowl, a washing stage is initiated with intravenous saline to a specific volume dependant on the bowl size. Washed red cells are then resuspended in a re-infusion pack ready for re-infusion (Figure 1). Reported red cell concentrations are approximately 50-80% (Allam et al., 2008; Kuppurao & Wee, 2010; Liumbruno et al., 2011). Internal RCH data which I have collected from vascular, obstetrics and orthopaedics have a concentration of approximately 50%, with a range 38 to 79% (Sullivan, RCH data).

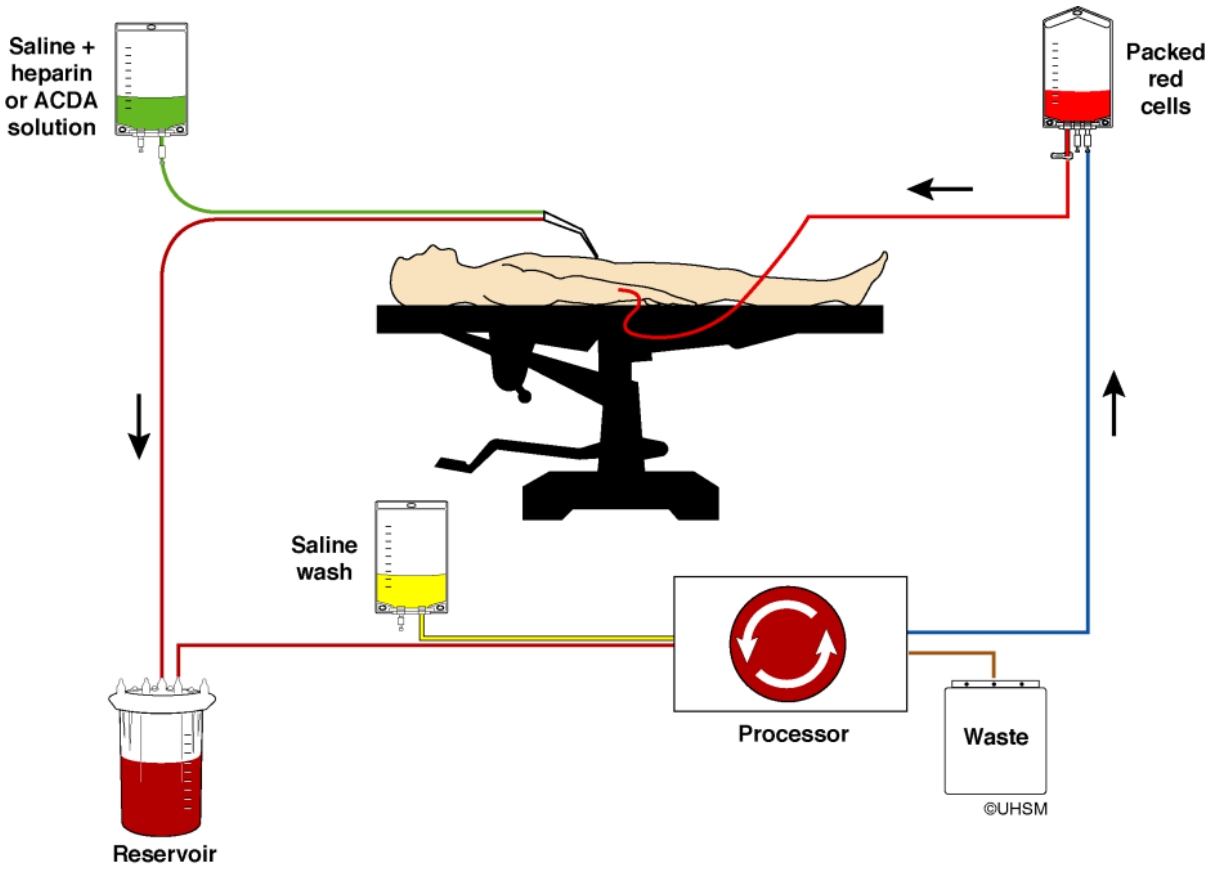

Figure 1: Schematic diagram showing the cell salvage process (reprinted with permission of the UK Cell Salvage Action Group).

Cell salvage is now routinely employed in vascular, orthopaedics, neurology and cardiac surgery to name a few (Allam et al., 2008; McDonnell et al., 2010). Multiple studies have found autologous blood to be a safer alternative to allogeneic blood; one such example is a five-year retrospective study by The Cleveland Clinic which found the incidence of adverse effects with autologous transfusion to be 0.027% compared to 0.14% with allogeneic blood (Domen, 1998). Table 1 summarises the benefits of cell salvage autologous blood.

Table one: Advantages of autologous cell salvaged blood

No risk of ABO incompatibility
Acceptability by Jehovah's Witnesses (dependant on their exact beliefs)
Blood is at room temperature
Less acidotic (allogeneic blood pH 6.8, autologous blood pH 7.6)*
Normal 2,3-DPG concentrations*
Normal potassium concentrations (0.3 mmol L^{-1}) *
Normal adenosine-triphosphate concentrations (Buys et al., 2017)
Available in cases where no allogeneic blood is routinely available due to antibodies, or insufficient time for full laboratory investigations

(*Unpublished data, Sullivan, RCH)

Intra-operative cell salvage can be of great benefit where there are issues supplying crossmatched allogeneic blood due to antibodies. It may be the only available source of blood in emergencies, as we have experienced (RCH unpublished work).

A key disadvantage of ICS is often thought to be that clotting factors and platelets, essential for maintaining haemostasis, are removed in the cell salvage washing process, and in cases of massive blood loss, ongoing coagulation testing must be undertaken to monitor plasma and platelet transfusion support. However, we have identified that autologous blood behaves differently to allogeneic, where it behaves more like whole blood. We have noted that patients who receive large volumes of autologous blood maintain haemostasis and require little to no additional clotting factors, suggesting that 1 mL of autologous blood is not similar *in-vivo* to 1 mL of allogeneic blood. Our ICS users have lower blood component support than those who

receive allogeneic only. Research into this *in-vivo* observation is currently in progress at RCH (IRAS: 225799; REC ref 17/NW/0586).

Autologous transfusion does have issues though. All adverse events or reactions should be reported to SHOT for ongoing investigations, monitoring and trending. There has been an increase in reports to SHOT with the use of cell salvage (Figure 2), but this could partly be due to its increased use and awareness to report to SHOT, although underreporting is still a SHOT concern (Haynes & Ralph, 2018).

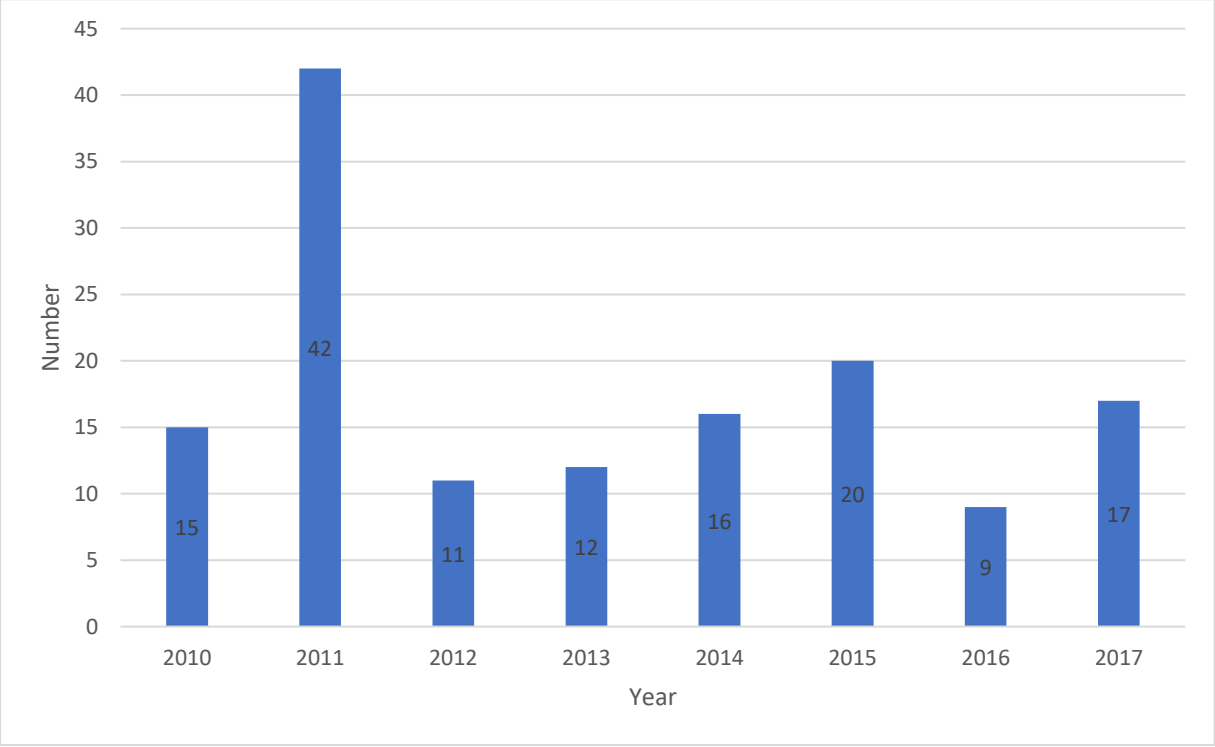

Figure 2: Cell salvage cases reported to SHOT

In the most recent SHOT report for the year 2017 there were 17 cases reported. There were no deaths or major morbidity reported due to cell salvage. Eight cases were from obstetrics, one from orthopaedics, two from trauma, one from urology, one from

vascular and four from spinal surgeries and emergency laparotomy. From these 17 cases, 14 were adverse events and three were reactions. Of the 14 adverse events, 12 were machine or operator failure/errors. These numbers should improve if cell salvage becomes employed more routinely and user experience and confidence is, therefore, increased. Of the other two cases, one was an administration error and the second was due to hyperlipidaemia. Cell salvaged blood was discarded. These last two were not directly caused by cell salvage but were identified whilst cell salvage was in use. Of the three clinical reactions, all three were in obstetrics. The first was an allergic reaction, but unlikely due to cell salvage as the patient stated that the reaction started before re-infusion. The other two were due to the use of a leucodepletion filter (LDF) resulting in hypotension. This will be discussed later.

CHAPTER 3 – OBSTETRIC CELL SALVAGE

For many years, and still ongoing to some degree, obstetric ICS has been contraindicated. This has resulted in these patients having to receive allogeneic blood as a treatment. The Royal College of Obstetrics and Gynaecologists (RCOG) 'Green-Top' guidance estimate that each year in the UK there are greater than 4000 cases of severe obstetric haemorrhage, with the majority receiving an allogeneic transfusion (Green et al., 2015). The most common cause of post-partum haemorrhage is uterine atony. At term, 600 mL min^{-1} of blood, equivalent to 20% of cardiac output, perfuse the uterus (Greenawalt & Zernell, 2017), so blood lost can be very significant at placenta separation.

A caesarean section (C/S) itself is a risk factor of needing a transfusion with reported rates varying from 1.4% (Sullivan et al., 2011) to 5% (Geoghegan et al., 2009). Caesarean section is the most common risk factor of abnormal placentation, with C/S rates in England increasing with 27.8% of deliveries in 2016-17 by C/S (NHS Digital, 2017). There is also an increase in the age of women delivering, by all methods, with a 9.5% increase in the 40 and over age group. Caesarean section rates increase with age and accounts for 44.1% of these women 40 and over. Of this, 23.5% were elective and 20.1% were emergencies. The 40 and over age group also have the highest body mass index at booking (NHS Digital, 2017). These are all risk factors for bleeding and can result in increased allogeneic transfusion.

With the endorsement of the Association of Anaesthetists of Great Britain and Ireland (AAGBI), the Obstetric Anaesthetists Association and The National Institute of Health and Clinical Excellence (NICE), obstetric cell salvage availability has been steadily

increasing and is now available in 84% of obstetric units, although only 50% of units have 24-hour access and use it routinely (Nelissen et al., 2018). The RCOG 'Green-Top' guidelines 47 and 52 (Green et al., 2015; Mavrides et al., 2016) recommend the use of ICS, although only for where the anticipated blood loss is great enough to induce anaemia or expected to exceed 20% of estimated blood volume. The most recently published AAGBI guidelines on cell salvage for perioperative blood conservation (published July 2018) did not recommend the routine use of cell salvage in obstetrics (Klein et al., 2018), however, their recommendations contradict each other. Intra-operative cell salvage is not recommended in routine C/S, however, it is when blood loss may exceed 500 mL (which is not uncommon in C/S) or if the patient is anaemic. It has been estimated that approximately 25% of pregnant women in Europe are anaemic, with this figure increasing in parts of Asia and Africa (Muñoz et al., 2017).

Despite these endorsements, cell salvage is still not routinely employed by most obstetric hospital units, essentially based on two concerns: amniotic fluid embolus and red cell alloimmunisation.

Amniotic fluid embolus (AFE) occurs in 1:8000 to 1:30,000 deliveries (Esper & Waters, 2011). It results in acute pulmonary hypertension, decreased ventricular output, cardiac and pulmonary collapse, altered mental state and disseminated intravascular coagulation (DIC) (Rogers et al., 2013). Precise elements in amniotic fluid (AF) that may cause the AFE, and the pathophysiology of the changes, are still not fully understood, although complement activation and mast cell activation may be involved (Benson, 2012). In the MBRRACE report (Knight et al., 2017), nine women died due

to AFE, equivalent to a mortality rate of 0.36. These were not linked to ICS. The clinical diagnosis of AFE is usually one of elimination and is now considered to be a type of anaphylactic reaction rather than an embolic disease, and perhaps would be better described as sudden obstetric collapse (Yentis, 1999).

The washing and filtering processes used in cell salvage have now been shown to effectively remove AF contaminants, fetal squames and other debris (Catling et al., 1999; Waters et al., 2000; **Sullivan et al., 2008**). It had been postulated that tissue factor, which is known to be present in amniotic fluid (Lockwood et al., 1991), may be the cause of the cardiovascular collapse and associated DIC that is seen in AFE. Tissue factor concentrations have been shown to increase in AFE (Conde-Agudelo & Romero, 2009). A 1997 cell salvage study showed no tissue factor present post washing stage (Bernstein et al., 1997). This study involved collecting all blood and AF into an older style cell salvage machine and then washed with no filtering. Since then, machine efficiency has significantly increased with improved washout rates. Squames cells were once thought to be a marker of AFE, but these have been found in healthy maternal circulation (Clark et al., 1986).

As for the second main concern of alloimmunisation, it is known that in obstetric ICS fetal red cells are collected during the cell salvage process. Due to the similarities between fetal and adult red blood cells in size and density, the cell salvage machines cannot differentiate between them, and, therefore, any fetal red cells aspirated will be washed, filtered and returned to the woman together with her own red cells. The significance of this contamination is not known and has not been systematically investigated. Does it increase the risk of alloimmunisation where the women produces

an antibody which could potentially have significant impact in future pregnancies due to HDFN? There is a need to be aware of the risk of formation of antibodies other than anti-D. Anti-D rates in pregnant women, although clinically significant, have been reduced by the use of routine prophylactic anti-D treatment throughout pregnancy. However, fetal hyperbilirubinaemia and anaemia in future pregnancies can still occur when antibodies have been formed to other mismatched red cell antigens, e.g. following the formation of anti-K, anti-c, anti-Fy(a) and anti-Jk(a) (Murphy & Pamphilon, 2008). This fact is not widely appreciated, and many investigators involved in obstetric ICS are still only concerned with the risk of anti-D.

It is unclear what follows if we re-infuse a larger volume of fetal cells into the maternal circulation than the amount that occurs secondary to transplacental haemorrhages in normal pregnancy. It is also unclear, at least in this setting, if there is a critical volume of fetal red cells required to cause alloimmunisation or if progressively larger volumes of fetal red cells increase the risk of alloimmunisation. Furthermore, there is no evidence to show how these rates of alloimmunisation compare to those following an allogeneic blood transfusion. As such, alloimmunisation is a significant concern.

These two areas of AFE and alloimmunisation have been the focus of research. The number of cases of ICS usage in obstetrics up to 1999 is sporadic with key larger reports being published between 1998 and 2000. Rebarber and colleagues (1998) published a multicentre cohort study involving 139 patients who had ICS compared to 87 similar control patients who had no ICS. They noted no significant difference between the two groups in length of stay, DIC rates, infectious morbidity or time on ventilatory support. However, there are many variables in this retrospective review in

that it was performed over three sites with different post-operative care and differences between the two groups.

In 1999, a report based on 10 patients examined for markers of AFE and alloimmunisation. Fetal red cells were found in all 10 samples, with up to 5 mL detected. Alpha-fetoprotein (AFP) was reduced to below detection limits in all 10 samples post wash. Squames cells were present in four cases pre-wash, with one still present post wash. Lanugo and vernix were not detected (Fong et al., 1999).

Also in 1999, Catling et al. published, which at that time, was a leading case series on 27 elective C/S cases where for the first time a leucodepletion filter (LDF) was included to investigate further reduction in the AFE risk. Patients were split into two groups, the first using one suction where all AF and blood was collected into the cell salvage machine, and the second using a separate suction initially to remove as much AF as possible to waste prior to blood collection. Samples were taken pre-wash, post-wash and post-filter and tested for AFP, fetal squames, trophoblastic cells and lanugo hair as markers of AF, and fetal red cells for alloimmunisation risk. Alpha-fetoprotein was reduced post-washing with no further reduction by the LDF. Fetal red cells were detectable but results were inconclusive and lacking reliability. Cellular elements (squames, white cells, trophoblastic cells) were not completely removed by the LDF. The authors concluded that “the risk of AFE is probably remote and should be carefully weighed against the life-saving potential of this technique” (Catling et al., 1999).

Following on from this, Waters et al. (2000) published a further report on 14 patients, including an additional step by taking a maternal sample from a femoral venous

catheter above the level of the uterine veins as they flow into the common iliac vein, in order to collect blood that may be contaminated with AF post-delivery. Only one suction apparatus was used, along with a LDF. They found that the use of a LDF (a newer advanced model compared to that used by Catling et al. (1999)) reduced levels of markers similar to those found in the maternal circulation.

From 2000 to 2005 there were no further published reports on investigating the risks, although there may have been further usage of ICS not reported. What these studies have shown is that there is limited information available before 2005.

In 2005 the National Institute for Health and Care Excellence (NICE) considered the evidence for the use of obstetric ICS and published guidance on its use (NICE Guideline, 2005). Their recommendations were based on review of three studies (Rainaldi et al., 1998, Rebarber et al., 1998, Catling et al., 1999), along with a further six case reports. In March 2007, the Health Service Circular "Better Blood Transfusion: Safe and appropriate use of blood" 2007/001 was published which required hospital trusts to implement a new programme of actions, which included to develop a blood conservation plan, reduce the need of blood and blood components, and of relevant to this area, progress further the use of cell salvage and avoid unnecessary blood transfusions in obstetrics (Health Service Circular, 2007).

Based on the recommendations, and the already active ICS use in other disciplines, our hospital wished to explore its use and suitability in obstetrics. Up to this time, ICS had only been used in orthopaedics and vascular, with much success, and obstetrics seemed the next logical progression. This led me in 2007 to undertake an exploratory

study (initially as part of my Master's Degree) into implementing ICS into obstetrics, and to investigate the concerns/contraindications previously mentioned with obstetric ICS. Despite having a low observed power, this study was one of the largest studies to date in the obstetric setting, and was published in 2008 (**Sullivan et al., 2008**). It had been recommended to use two suction devices to minimise the risk of re-infusing AF resulting in AFE (Rebarber et al., 1998; Fong et al., 1999, Potter et al., 1999) where the AF is initially collected by a separate suction to waste prior to collecting blood. There was no evidence to support this recommendation but based solely on a theoretical suggestion. To assess this theory, along with the AFE and alloimmunisation risks, 34 consented patients undergoing elective C/S were split randomly into two groups; the first group only used one suction apparatus to collect both AF and lost blood into the cell salvage collection reservoir, and the second group involved a second suction to remove AF to waste first prior to blood collection. Samples were taken pre-wash, post-wash and post-LDF, and tested for markers of AFE and alloimmunisation and heparin contamination. Seven cases were excluded as insufficient blood was lost/collected to process (two from group one and five from group two). Alpha-fetoprotein concentrations were significantly reduced post-wash ($p < 0.001$) with no further reduction seen post filtration ($p = 0.996$). Fetal squames were present in all post-wash samples, but only present in two cases post filter (both group one). Heparin was washed out in all cases. We identified that the final re-infusion volume was higher in those in group one (i.e. one suction), as was the haemoglobin/haematocrit concentration, however, both were not statistically significant. Based on this, we therefore suggested that only the one suction device should be used. Apart from the financial savings of using one less instrument, practically it is easier to collect everything into one and not having to swap halfway

through surgery. The other advantage is that there is no clear point where it is only AF and then blood, so by using two suction devices the volume of salvaged red cells will be reduced, resulting in reduced return to the woman. We showed that the washing and filtering process significantly reduced concentrations of AF contaminants and that whether the squames cells are of maternal or fetal origin is irrelevant as the filters remove both. We concluded that one suction may be used, a LDF should be included and partial bowls should not be re-infused. It was hoped that these results could be used to change the guidelines and allow routine use of ICS as opposed to overriding guidance in extreme emergency. It was not the intention in this study to re-infuse any ICS blood, however, approximately 500 mL was re-infused to two cases, which shows that even in elective C/S, significant and unexpected blood loss can occur.

Since our leading report, the number of hospitals now only employing one suction device has increased. A correspondence reply in 2010 confirmed our recommendation that there is no evidence to support two suction devices (Catling et al., 2010). An audit from 2014 reported that 12% used one suction only (UK Cell Salvage Action Group, 2014), but in 2017 this increased to 58.1% (Khan et al., 2017), showing there is a clear indication that more users are moving to one suction.

There have now been over 2000 reported cases of re-infusion in obstetrics with no reported adverse events or AFE linked directly to ICS (Rebarber et al., 1998; McDonnell et al., 2010; Sullivan et al., 2011; Khan et al., 2017; Akindele et al., 2018; Kenyon et al., 2018; Orr & Wrench, 2018; Yan et al., 2018; Zeng et al., 2018; **Sullivan & Ralph, 2019**). Reports have shown a shorter length of stay, lower hysterectomy rates and reduced allogeneic transfusion rates when ICS has been employed (Zeng

et al., 2018). This number will carry on increasing as further studies are published. It is now widely agreed that AFE is no longer a valid contraindicative reason, and this risk remains entirely theoretical. It has been reported that all recently delivered women must have AF in their blood and, therefore, if there was AF in the returned ICS blood, it would not represent an increased risk (Catling & Joels, 2005).

In 2019, I published a key report on the last ten years of our experience (**Sullivan & Ralph, 2019**) where we have re-infused to 1170 women, all without any clinically significant adverse events and no cases of maternal collapse or hypotensive episodes, with and without LDFs. This is now the largest published report to date. I presented an update on the use of ICS, data on alloimmunisation rates, quality of cell salvaged blood and the role of partial bowls. To my knowledge, there is no other such comprehensive report and it is hoped it will be used to lead further research, be of assistance to other users, and be used to rewrite guidelines. Intra-operative cell salvage methodology was included, alongside quality control processes, and following up women post re-infusion. I concluded that the risk of amniotic fluid embolus should not be a barrier to implementing an ICS service. In contrast to our initial 2008 report, we now recommend that LDFs are no longer used in obstetric ICS, based on observational data.

Alloimmunisation is still however an ongoing concern though but there has been limited investigations on this, with case numbers varying from 3 to 27 (Rainaldi et al., 1998; Catling et al., 1999; Fong et al., 1999; Waters et al., 2000; McDonnell et al., 2010). Fetal red cell contamination is one of my big interests within my profession, and as such, I therefore keenly investigate this area. The only way to confirm if there is an increased risk/rate of alloimmunisation due to cell salvage is to know what

concentration of fetal cells are in the final product, and to follow-up all women post re-infusion. These have both been addressed. Of the 1170 women re-infused in our hospital, 509 (44%) have provided a sample three to four months post-delivery, and on further checking of the women who did not provide a sample, a further 138 had a sample taken at a later date for other reasons (such as subsequent pregnancy, other surgery/procedures) taking the total to 647 (55%). Of these, two cases (0.3%) had returned a positive antibody screen post ICS. Both were anti-E. The first case was an abruption with an estimated blood loss of 600 mL, receiving 237 mL ICS blood and one unit allogeneic transfusion (unit was E-antigen negative). The second case was a twin vaginal delivery requiring urgent surgical intervention due to complications and bleeding with the second baby being born by caesarean section an hour later. Four hundred mL ICS blood was re-infused. No allogeneic units were transfused. The techniques used within our laboratory have a similar detection profile to enzyme methods for Rh antibodies, and as such these antibodies may be 'naturally occurring' (i.e. not as a result of red cell stimulus), but this cannot be proven with confidence.

But how do these rates compare to normal pregnancy/normal alloimmunisation rates? Answering these questions, will assist in answering the alloimmunisation concern.

I investigated and published a report on "Obstetric intra-operative cell salvage and maternal fetal red cell contamination" (**Sullivan & Ralph 2018**), where fetal red cell levels in women immediately prior to delivery and post-delivery were quantified and compared to those found in cell salvaged blood, as well as investigating antibody formation rates following allogeneic transfusions. I identified that with our results and that of others, up to 78% of women are exposed to fetal red cells ante-natally with

levels up to 5.15 mL, and more than half of all mothers are exposed to fetal rbcs post-delivery with levels up to 26.6 mL. These volumes are comparable to those found in ICS. We have not noted an increase in antibody formation within our population when compared to normal pregnancy or those having an allogeneic transfusion, although these numbers (647) are still too small to answer this risk with confidence.

From data I have collected within our hospital at RCH, I have calculated the current risk of producing an antibody due to allogeneic blood transfusion is 0·35%. To rule out an increased rate of producing an antibody from receiving ICS blood with 95% certainty, that the upper 95% confidence interval is less than 0·45%, would require a total study size of 15,236 patients; the total sample is split randomly, 7618 patients to receive allogeneic blood compared to 7618 receiving ICS blood, making this study not feasible. We are the only hospital who follow up women, and we are strongly recommending that other institutions follow-up women three to four months post-re-infusion in order to collect data on alloimmunisation rates. We recommended to a large proposed randomised controlled trial in 2013 to follow-up women, which would have significantly increased the numbers, however, it was decided by the research team to not assess alloimmunisation at that time (personal communication). Other hospitals are now following our practice and starting to follow-up women for antibody formation. In one UK hospital, referencing our work and using it as a foundation, 225 women had an ICS re-infusion. Of these, 52 (23%) had an antibody screen. No antibodies were detected, concluding that ICS is not associated with alloimmunisation (Akindele et al., 2018). Another hospital, who I assisted in their report based on our work, followed up 80 women, for which none had produced an antibody (Davies et al., 2018). I am now

working with this hospital on setting up a formalised follow-up system. I am also working with another hospital to follow up their women who have had a re-infusion.

It is hoped that other centres will adopt this approach, and I would work with others to set up a central database to capture all follow-ups. I am in consultation with several hospitals to repeat the fetal red cell levels in maternal circulation pre- and post-delivery, using more sensitive methodologies and a significantly higher sample size population.

However, despite the evidence listed, a recent large randomised controlled trial on cell salvage during caesarean section 'SALVO' has somewhat negatively impacted on the use of ICS in obstetrics based on alloimmunisation concerns (Khan et al., 2017). Their results have been adopted into the recently published AAGBI guidelines where they have recommended to not use ICS routinely (Klein et al., 2018). We have responded to SALVO with our concerns (**Ralph & Sullivan, 2018**), and the AABGI guidelines, as have others.

In the SALVO trial, it was reported that fetal-maternal haemorrhage (FMH) was significantly increased by the use of cell salvage, however, the SALVO authors do state that results should be treated with caution due to such small numbers and data available. Data are only available on those cases where the FMH was greater or equal to 2 mL, and a RhD positive baby was born to a RhD negative mother. From 761 re-infused cases, this FMH risk was based on 30 cases: 21 in the ICS group and 9 in the non-ICS group. Of the 21 cases investigated for alloimmunisation in the ICS group, six (29%) did not have a re-infusion but the data compared included these six, and so

raises concerns with the validity of the data. Of interest, the non-ICS group had the highest fetal contamination (37 mL) compared to 26 mL in the ICS group. It cannot be postulated that collecting ICS blood only increases the number of fetal cells entering the maternal circulation. It is concerning that such important decisions have been made on such a small sample size, where there are more significant sample sizes studied, including our data from over 180 cases, the largest to date by far. I am in correspondence with the trial authors to discuss this finding.

The use of ICS in obstetrics had been increasing. In 2014 the UKCSAG repeated a survey of UK hospitals using ICS and compared results to the 2010 survey (UK Cell Salvage Action Group, 2014). Most specialities have seen similar levels of use between the two dates, however, there has been an increase in its use in obstetrics and gynaecology. In 2014, obstetrics and gynaecology accounted for 25% of ICS use, the largest of all specialities (Figure 3). It was postulated that this obstetric increase may be due to the SALVO trial recruitment. However, as more information is becoming readily available and support is available, this could also have contributed to its increase. Unfortunately, one hospital has commented that their use of ICS and enthusiasm has reduced post-negative SALVO findings (Orr & Wrench, 2018).

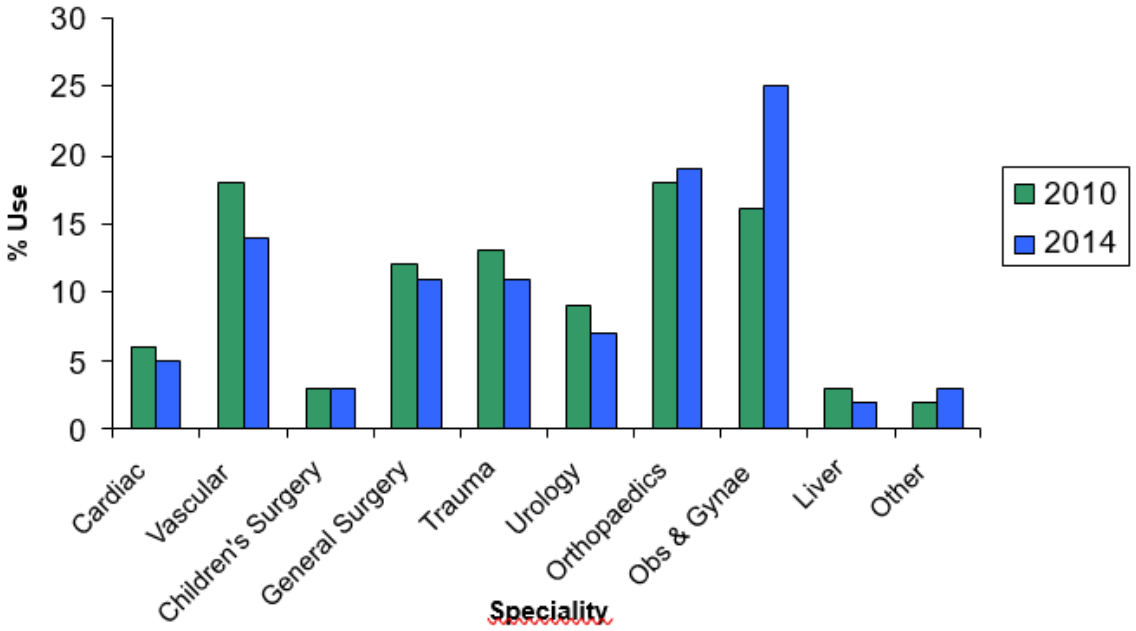

Figure 3: Cell salvage use across the UK (taken from the UK Cell Salvage Action Group survey, 2014)

Since 2008, I have carried on investigating and improving our ICS service offered. Apart from concerns with AFE and fetal red cell alloimmunisation, concerns have been raised about staffing and finances to run an ICS service in general. The 2014 UKCSAG survey (UK Cell Salvage Action Group, 2014) identified that 21% of hospitals were unable to use ICS out of hours, compared to 23% in 2010, so no significant improvement. Reasons why not used included lack of trained users and insufficient staff available. In 2011 we published a report on staffing, finance and implementation of the service **(Ralph et al., 2011)**.

In this study, blood was collected and re-infused to 70 women. We assessed the quality of the returned blood by taking a sample and testing the haemoglobin and haematocrit and assessing fetal red cell contamination. Mean haemoglobin concentration was 142 g L^{-1}, and mean haematocrit was 0.418 L L^{-1}. All samples had

detectable fetal red cells with a range 0.2–12.9 mL. Throughout this period, we investigated allogeneic transfusion rates and noted a reduced allogeneic transfusion rate over a three-year period.

To overcome staffing and training barriers, ICS is included into routine preparation of our obstetric theatre and is entirely run by the normal quota of theatre staff. We also introduced a competency training programme for operating department practitioners. The operating department practitioner operates the cell salvage machine as well as assisting the anaesthetist. No additional staffing has been required, and at the time of reporting (2011) ICS was being used for more than 40% of C/S. This figure is now greater than 99% in 2018 (**Sullivan & Ralph, 2019**). This showed that it is possible to implement an ICS service with no additional staffing requirements, which is often seen as a limiting factor. We have since been contacted by other hospitals to ask our advice on how to implement ICS.

In our initial 2008 report we recommended the use of a LDF, partly based on historical concerns and comments, our lack of experience in obstetric ICS, and the NICE 2005 guidance which recommended that they could be considered (NICE Guideline, 2005). Before current LDF introduction, several hundred patients received ICS blood with no filter or less effective models, with no evidence of AFE (Catling et al., 2010). The washing process alone has now been shown to completely remove AF and reduce fetal squames cell levels (Thornhill et al., 1991; **Sullivan et al., 2008**). In the SALVO trial (Khan et al., 2017) only 54.1% of 26 UK obstetric units in the trial used LDFs, demonstrating a move away from its use in obstetrics, partly due to concerns with hypotensive reactions and slow re-infusion rates (Khan et al., 2017; Haynes & Ralph,

2018). Since SHOT started recording cell salvage events in 2010 there have been 20 hypotensive events due to the use of the LDF (Haynes et al., 2017). Due to concerns and comments with the filters, we wrote a correspondence (**Ralph & Sullivan, 2010**) discussing their use and confirming that the manufacturers had not validated their use for its ability to remove fetal squames cells, but rather that they only remove white cells, fat and microaggregates. Within our hospital we stopped using the filters in 2015, and since then we have not had any adverse events due to not using these filters, which led us to recommend to not use LDFs in our recent 2018 paper. The recent published AAGBI guidelines only recommend that LDFs should be considered for cancer surgery (Klein et al., 2018).

Also, in our initial 2008 report we recommended to not re-infuse partial first bowls. The role of partial bowls is now a debated subject, although evidence for this concern is limited and could just be theoretical based on the hypothesis that “partially filled bowls are inadequately washed based on the physics of centrifugal cell separation” (Szpisjak, 2001).

Since then I have assessed the quality of the blood obtained from partial bowls (defined as a first bowl that does not fill after processing in the automatic setting on the machine) (**Sullivan & Ralph, 2019**). The haemoglobin and haematocrit are lower in partial bowls, as would be expected, but the plasma free haemoglobin, lactate dehydrogenase (LDH) and potassium concentrations from full bowls were found to be higher ($p < 0.001$), with albumin levels similar with no significant difference between full and partial bowls ($p = 0.861$). Plasma free haemoglobin and LDH concentrations from partial bowls were found to be similar to that of allogeneic blood, however, levels

from full bowls were higher than allogeneic ($p < 0.001$), consistent with our previous finding that there is more haemolysis in ICS compared to allogeneic blood (**Sullivan & Faulds, 2014**). I concluded that the quality of blood processed from partial first bowls is no worse than that of full bowls, apart from haemoglobin/haematocrit, and that partial first bowls should be considered in obstetrics for re-infusion. Haemonetics® product literature now states US Food and Drug Administration (FDA) clearance to wash and re-infuse a partially-filled bowl to maximise red cell re-infusion (Haemonetics® product brochure, 2012), although information available to support this is limited.

One consideration is how partial a bowl will be cost effective? For example, does 115 mL blood (from a 125 mL bowl) with median haemoglobin of 98 g L^{-1}, haematocrit 0.286 L L^{-1} reduce the allogeneic transfusion risk? The re-infusion of even these small quantities of blood could be of benefit to women who refuse blood products, such as Jehovah's Witnesses, where all salvaged red cells could be potentially returned to the patient. At the RCH, some women had ICS blood collected but were not re-infused as the processed volumes were only partial first bowls. A minority of these cases went on to receive an allogeneic transfusion, although it is unclear whether it was due to a low starting haemoglobin, poor tolerance of the small blood loss during delivery or continued bleeding post-natally that resulted in the further drop in haemoglobin. If partial bowls were re-infused, potentially the allogeneic transfusion rate could be reduced further, something that could be audited if we introduced partial bowl re-infusions.

Another debated point, which authors often contradict themselves frequently, is whether cell salvage should be employed for all cases, or just for those with expected high risk/high blood loss cases. Within our hospital we collect on all cases, setting up a ‘stand-by’ system, also known as ‘collection only’, where all blood lost is collected into the cell salvage reservoir. If blood volume collected is significant to process fully, the more expensive processing kits are opened at this point. This method has been shown to be cost effect (Waters et al., 2007; Sikorski et al., 2017; Zeng et al., 2018), and we have not noted a significant increase in costs associated with this practice.

Since 2012, the number of cases where ICS has been used to collect blood within our hospital has raised from 95.4% to 99.1% in 2017. Not all cases lose sufficient blood to be processed, but the percentage of blood re-infused of those processed cases has raised from 53.1% in 2012 to 70% in 2017. Many women who receive cell salvaged blood are women who have no pre-operative predictors for blood loss. By trying to predict, and only collect for those women at risk of bleeding, many women who bleed will be missed, and limiting the use of ICS to high-risk cases may result in reduced opportunities for training and maintenance of skills (King et al., 2009). I have shown there is no correlation between C/S category and ICS volume, C/S category and estimated blood loss, nor para gravida and estimated blood loss (*Spearman’s rank* correlation 0.253, -0.228 and -0.050, respectively) which confirms the perception that those women at risk of bleeding cannot be predicted. There was weak correlation between estimated blood loss and length of stay (0.334), and moderate correlation between estimated blood loss and ICS re-infused volume (0.535), which would be expected.

Although each year the percentage we process to re-infuse increases, it is unlikely more than 80% of processed collections will be re-infused as some will always be wasted due to training, partial first bowls and rarely, women who decline the re-infusion.

CHAPTER 4 – ALLOGENEIC TRANSFUSION RATES POST CELL SALVAGE

INTRODUCTION

As well as recording data on ICS use, we have also been auditing allogeneic transfusion rates. All obstetric women who receive an allogeneic transfusion are audited to assess reason why transfusion given and whether it was appropriate or could have been avoided. The use of ICS has been critical in reducing these rates, although it is not the sole cause. We have an active patient blood management programme in place, which includes treatment of ante-natal and post-natal anaemia with total dose intravenous iron (implemented 2012), tranexamic acid for major obstetric haemorrhage, and education and training of medical staff in using blood wisely. As can be seen in Figure 4, the number of cell salvage re-infusions has increased, and the number of allogeneic transfusions has reduced.

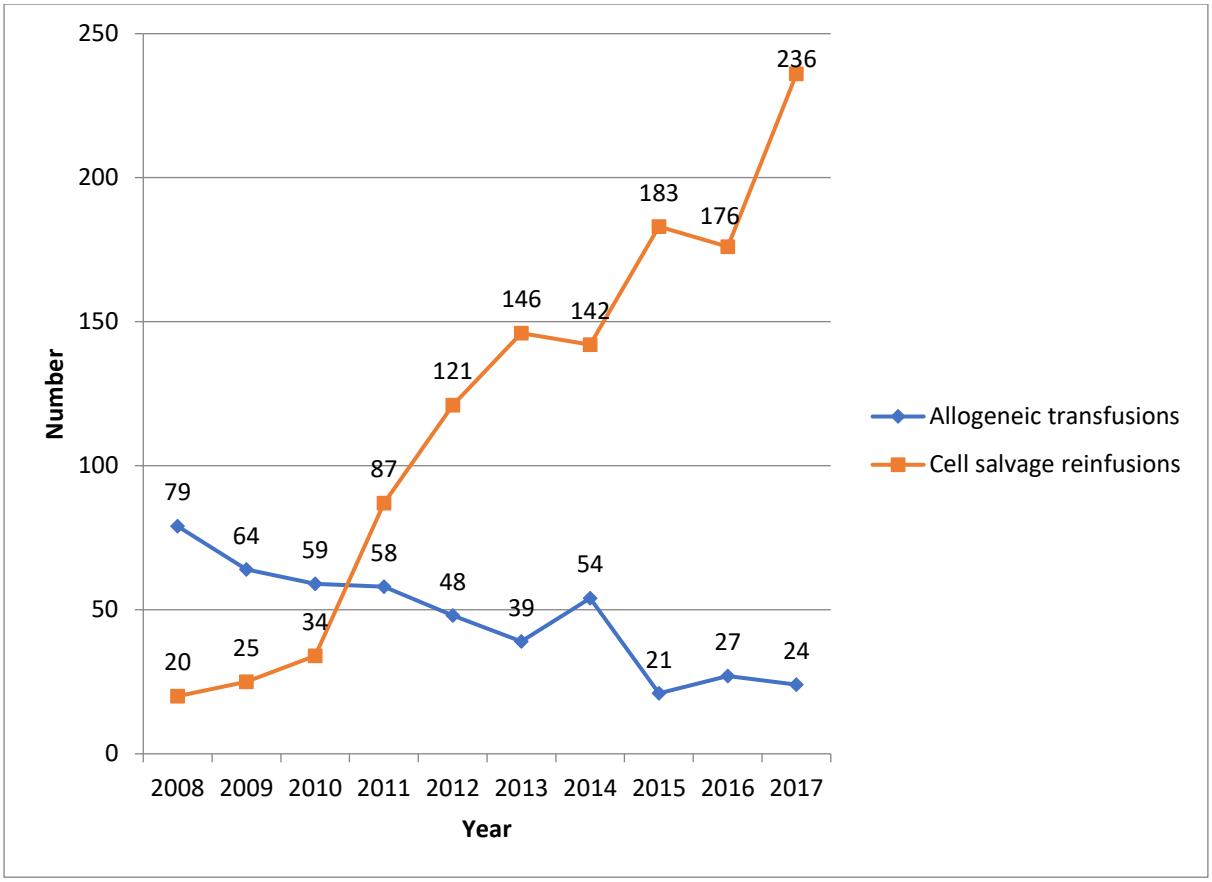

Figure 4: Number of women transfused allogeneic blood or cell salvaged blood

The rate of allogeneic transfusions in our obstetric population has fallen to 0.6%, which includes ante-natal and post-natal women who have both vaginal and C/S delivery, one of the lowest in the UK. The allogeneic transfusion rate for those women who delivered by C/S is 0.4%, far lower than the suggested 5% standard in the UK (Geoghegan et al., 2009). Not only has the percentage of women needing a blood transfusion dropped to below 1%, but as important, is the reduction of the number of units of blood transfused, which has reduced from average 3.3 units pre-2008 to less than two units after 2012 when the service was fully established. Obstetric patients (C/S and vaginal deliveries) now account for 0.3% of allogeneic red cells units transfused within our hospital. I have a responsibility to manage and improve

transfusion services within our Trust and the NHSBT, where I sit on several working groups.

Since 2016 the allogeneic transfusion rate has plateaued, mainly being due to women requiring blood following vaginal deliveries. As can be seen in Figure 5, the majority of our allogeneic blood transfused goes to those women who deliver vaginally. The rate in this group is increasing, with the rate in the C/S group falling.

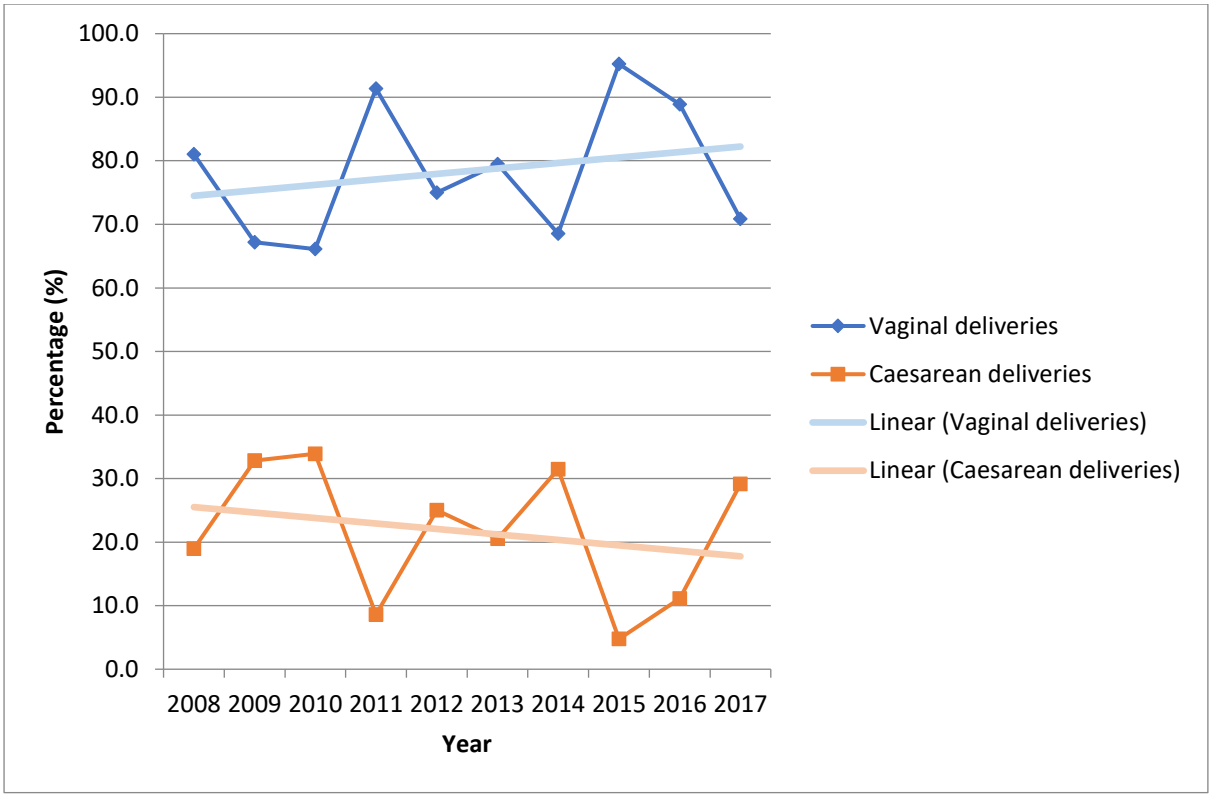

Figure 5: Percentage of allogeneic transfusions – vaginal or caesarean deliveries over time

In 2017, 71% of allogeneic blood use in obstetrics went to women post vaginal delivery. As part of our patient blood management programme, this is something that needs to be targeted.

CHAPTER 5 – VAGINAL CELL SALVAGE

As has just been shown, the biggest users of allogeneic red cells within our obstetric department are now those who deliver vaginally. Could ICS be used within vaginal deliveries?

The use of ICS in vaginal deliveries has been contraindicated mainly because of bacterial contamination concerns, although there is no evidence to support or reject its use. Concerns also lie with the collection and the quality of returned blood. This led us in 2014 (**Teare et al., 2015**) to undertake a feasibility study into whether blood lost vaginally can be safely collected, processed and re-infused after vaginal delivery. This was the first study to attempt to collect vaginal blood loss. A project grant was awarded from the National Institute of Academic Anaesthesia to support this. Fifty women were consented and enrolled. No women were re-infused.

One of the first challenges was how to collect lost blood and stop it from clotting. An under-buttock drape with pouch was used and 200 mL heparinised saline was run rapidly into the pouch, and then adjusted to a slow drip. Blood collected could then be aspirated into the cell salvage machine. Samples were taken pre- and post-wash, and post LDF filter and tested for quality, fetal red cell contamination and bacterial contamination. To assess the risk of bacterial contamination, blood cultures were collected from 20 post-wash samples from emergency C/S cases.

Of the 50 cases, results were available from 34 cases. The remaining 16 were not included as were taken from a partial bowl, and at that time, the quality of partial bowls was not known. Alpha-fetoprotein as a marker of AF was significantly reduced post-

wash, as was heparin, albumin, LDH and plasma free haemoglobin. Fetal red cells were present in all cases. The results obtained were consistent with our and other's experiences with ICS and C/S. The main additional concern still remaining was bacterial contamination.

From all 50 cases enrolled, bacteria were detected in all cases post-wash and post-filter, with a significant reduction in concentration by the washing process. The predominant bacteria identified were *Escherichia Coli*, *Enterococcus species.* and coagulase-negative *Staphylococci* which were likely to be part of the genital or skin flora. From the comparison group of 20 C/S cases, all had detectable bacteria, with contamination levels from vaginal similar to those from C/S. We calculated that the median dose that could potentially be re-infused was similar to dental procedures and is likely to be clinically insignificant. We concluded that salvaged vaginally collected blood was unlikely to increase the risk of infection, but this has not been established.

We have since collected on a further three cases but none have been re-infused. The 34 cases reported is still the largest report to date. In an editorial in the International Journal of Obstetric Anesthesia (Wilson & Wrench, 2015), reference was made to our vaginal study and how the findings add "to the existing evidence challenging this concept".

Our work and input was used in a later report by Lim and colleagues (2018a) who in 2017 assessed the use of ICS after vaginal delivery. Re-reviewing their cases they identified ten women had had a cell salvage re-infusion post vaginal delivery. A further 28 had blood collected but insufficient volumes for processing. A second group of

vaginal deliveries with no cell salvage re-infused were compared. Results showed no difference in length of stay between the two groups, and there were no cases of wound infection, sepsis, thromboembolic events, AFE or increased rates of allogeneic transfusion, however, the numbers (10) are very low to make conclusive decisions. Since then, there have been a handful of other reports involving cell salvage of vaginal blood lost following vaginal deliveries, but numbers still limited. In one case 380 mL of vaginal lost blood was collected and re-infused to a Jehovah's Witness experiencing a significant PPH. The woman showed no signs of post-natal sepsis, and blood cultures were negative after 48 hours (Weingarten et al., 2017). I have received other personnel communications, for which I am encouraging these users to publish.

Further large-scale trials are required to confirm the safety and effectiveness, compared to allogeneic blood, which we recommended in our 2015 study. A survey was undertaken by Nelissen and colleagues (2018) to assess the use of ICS in obstetrics including after vaginal delivery, and perceived thoughts on its use. There were no hospitals in England, Scotland or Wales who use ICS routinely after vaginal birth, although there was support for its use.

Based on our results and experiences, within our Trust policy on cell salvage, we state that if surgical intervention is attempted vaginally, ICS may be considered in life threatening circumstances (when there are no alternatives, such as with Jehovah's Witnesses). We are currently re-reviewing our use of ICS and hope to work in collaboration with others to advance this area.

CHAPTER 6 – QUALITY OF CELL SALVAGE BLOOD

Another key area that I am focusing on, which currently has not been investigated in much depth, is the quality of blood being returned to the patient. Is it acceptable to return blood without even knowing exactly what is in it? How do we know that what we are routinely returning is of safe and consistent quality? This area I feel needs to be investigated.

Quality control (QC) is seen as integral in laboratories but not so in theatres. In the 2017 SHOT report, the authors commented that “an awareness of cell-salvaged blood quality is important for both operators and clinicians”. They recommended that cell salvage operators should be trained and competency assessed with ongoing retraining and updating, standard protocols should be in place and the quality of the re-infused product known (Haynes & Ralph, 2018). The UKCSAG 2014 survey identified that 55% do not control the machine and 61% do not QC the users, with no improvement since the 2010 survey when 58% do not QC the machine and 68% do not check the user (UK Cell Salvage Action Group, 2014).

I have an active interest in ICS quality control/assurance and have undertaken various studies to investigate the implementation of a readily available, cheap, quick and reliable testing system for hospitals to assess the quality.

Heparin is the anticoagulant used in all cell salvage specialities within our Trust. There was no cheap quick screening test readily available to detect heparin contamination in ICS. There had been concerns from the surgeons that heparin may still be present in the final end product which could compromise the patient’s recovery. Previous

studies have shown that heparin is still present in the final product (Umlas & O'Neill, 1981; Kling et al., 1988; Burman et al., 2002), but in our 2008 study we showed it is possible to completely remove heparin. To ensure that the cell salvage machines wash out the heparin to a safe level, I devised a suitable test. Current screening test is the anti-Xa assay, but this is expensive and not readily available in all laboratories. It requires specialised reagents and time to make these reagents, calibrate and QC the analyser.

The standard laboratory thrombin clotting time test was modified and shown to be a reliable and sensitive marker in measurement of heparin up to 0.14 iu mL^{-1}. This modified test was only designed to exclude gross heparin contamination. Anything above 0.15 iu mL^{-1} are flagged as high heparin concentration and require further testing. Heparin concentrations greater than 0.5 iu mL^{-1} affect *in-vivo* coagulation, so at concentrations up to 0.14 iu mL^{-1}, this equates to a clinically insignificant amount that could be re-infused safely. This was published in 2012 (**Sullivan et al., 2012**).

This QC test has been successfully incorporated into our hospital QC scheme for obstetrics, vascular and gynaecology, but not for orthopaedics. This being due to high levels of haemolysed blood in orthopaedic cases post wash, and the coagulation analysers unable to test grossly haemolysed samples. The haemolysed samples obtained were of concern, especially with the effect of re-infusing haemolysed blood to patients. This is still a reoccurring theme in ICS with further haemolysis caused by the suction pressure, washing/centrifuging process and/or the aspiration method, but the significance has not been fully addressed, and so I investigated the role of haemolysis in ICS.

Blood lost from orthopaedic surgery tends to be of a gradual loss, with blood suctioned from bone and tissue in a ‘skimming’ fashion, resulting in mechanical trauma, whereas in obstetrics and vascular surgery there is more of a ‘pooling’ of lost blood. When these cells are collected they are not exposed to the same trauma as that in orthopaedics. Free haemoglobin as a result of haemolysis released into the circulation is bound by haptoglobin which is then removed by the reticuloendothelial system. When in excess, or insufficient haptoglobin to bind to the free haemoglobin, free haemoglobin becomes nephrotoxic resulting in circulatory free haemoglobin in the plasma. When levels exceed reabsorption rates in the kidneys it presents in urine as haemoglobinuria (Dacie & Lewis, 1991; Rother et al., 2005). Acute renal failure could theoretically occur in severe episodes of haemoglobinuria caused by re-infusions of haemolysed ICS blood. This, however, does not seem to occur in the clinical setting possibly due to the low volumes returned in most surgical collections.

In addition, the haem part of haemoglobin breakdown can increase procoagulant effects resulting in increased platelet activation and increased release of inflammatory substances (Rother et al., 2005; Sloan et al., 2009). Damaged cells also cause coagulation abnormalities and other inflammatory morbidities including acute lung injury (Kelleher et al., 2011).

Free haemoglobin is also a scavenger of nitric oxide irreversibly binding to and reducing levels. Nitric oxide is a regulator of smooth muscle tone and platelet activation and acts as a vasodilator in the microcirculation (Rother et al., 2005; Ley et al., 2012). Reduced levels result in vasoconstriction, systolic and diastolic blood

pressure increases, reduced tissue oxygenation, smooth muscle dystrophy, gastrointestinal contractions, endotheliac dysfunction and platelet aggregation with increased thrombotic events (Rother et al., 2005; Sloan et al., 2009; Kelleher et al., 2011; Vermeulen Windsant et al., 2012). Haemolysis also results in the release of enzymes that can affect nitric oxide synthesis (Rother et al., 2005).

With all these potential consequences, I undertook a project to quantify the amount of haemolysis in orthopaedics (skimmed) and obstetrics (pooled) and to compare that to allogeneic blood. This was published in 2014 (**Sullivan & Faulds, 2014**). I confirmed the hypothesis that haemolysis (as measured by free haemoglobin levels) was significantly higher in orthopaedics compared to obstetrics and allogeneic blood. This does not, however, address the clinical significance of re-infusing haemolysed blood. *In-vivo* effects, dilutional effect and haptoglobin levels for example need to be considered, although I feel there is no significance due to the thousands of re-infused cases reported with no complications due to haemolysis. I did review the notes from the 50 patients enrolled into this study and noted no new cases of renal dysfunction or other complications. This study needs to be repeated, including assessing the patient's free haemoglobin levels pre and post ICS re-infusion, as well as testing haptoglobins and monitoring renal function.

Following on from implementing a heparin screening test into our QC package, I also wanted to ensure there was no gross haemolysed blood being returned, and so next investigation was for a cheap reliable and sensitive test for haemolysis. The gold standard test would be plasma free haemoglobin levels, but this requires expensive devices and consumables that are not available to many hospitals. Therefore, I

explored the possibility of using the LDH test as an alternative. Lactate dehydrogenase is an enzyme widely distributed in almost all body cells and when cells are damaged, it is released into the circulation and can therefore be quantified. In 2013 I published results showing the sensitivity and reliability of this test (**Sullivan & Faulds, 2013**). The LDH test strongly correlated (*Spearman's rank* correlation 0.960) with plasma free Haemoglobin levels measured by a specialised HemoCue® Plasma/Low Haemoglobin System (HemoCue®, Ängelholm, Sweden). Other hospitals have used potassium as a haemolysis marker, so I also compared potassium to plasma free haemoglobin levels and concluded the correlation was not acceptable (*Spearman's rank* correlation -0.16) and recommended that potassium should not be used. This LDH test has since been incorporated into our QC package alongside heparin test, haemoglobin/haematocrit and albumin (microalbumin). Other hospitals have been in contact with myself enquiring about our findings/experiences.

Although we have suitable tests available, acceptable ranges for quality markers have not been established. In 2010-11 on behalf of the UKCSAG we asked hospitals to participate and provide data on results from QC sample testing. Uptake was poor with insufficient data collected to answer this question. I presented this at British Blood Transfusion Society annual conferences to gain interest (**Faulds & Sullivan, 2011**; **Faulds & Sullivan, 2013**). It is hoped that the recently reformed UKCSAG will repeat this, for which I am currently working in collaboration with to undertake a survey on what QC hospitals currently do, how often, and if they would be interested in setting up a national database in order to establish reference ranges. In the meantime, I am currently setting up a local database.

Several users still report quality as a percentage, for which I disagree with and I am promoting an alternative method of reporting quality. Reporting the washout rates of the machines as a percentage does not provide useful clinical information for the clinical team. Rather than knowing that the average washout rate is 98% for proteins, would it not be more beneficial to know exactly what concentration is in the final product (e.g. 1 mmol L^{-1}). Again, it is hoped that in collaboration with the recently reformed UKCSAG group this can be taken forward.

Another challenge for the future is to make QC testing point of care so results are immediately available, rather than waiting for laboratories to process and report. This I will be exploring further.

Quality control is now part (*ad-hoc*) of our Trust protocol but I am currently drafting a Trust policy on quality assurance to formalise and standardise QC along with servicing, down-time reports, training, documentation and competency assessment.

CHAPTER 7 – FUTURE

Now that ICS has become established within our hospital for caesarean sections, the focus has been moved to the quality and consistency of blood returned, and general quality control including machine downtime logs, staff training and competency. Collection and cell salvage of vaginal blood following vaginal delivery is another specific area for development.

Outside these two topics, other general issues need to be reviewed.

In an ever-demanding NHS setting, hospital budgets are tightly controlled, and expenditure must be justified. Is ICS cost effective to use in all surgical cases or limit to high risk cases where massive blood loss is expected? It had been previously reported that once the capital of the cell salvage machine had been paid for, ICS was cost neutral so long as the equivalent of two units of blood had been transfused, and any more than this is a cost saving (Waters et al., 2011). However, more recent reports have suggested that ICS is no longer cost effective in routine C/S (Khan et al., 2017; Lim et al., 2018b).

Recommendations to not use ICS routinely based on cost alone is worrying and could be a backwards step in implementing this critical service. Assessing costs must accurately reflect clinical practice in which collection costs are considered separately to costs of processing and providing a re-infusion. We have previously shown it is possible to run a service with no additional staffing needed, and in a recent report, significant cost savings were made by the use of ICS. From 543 C/S cases in a one-year period, cell-salvaged blood was re-infused to 182. Based on a theory that one

unit allogeneic blood is the same as one unit cell-salvaged blood, a cost saving of £9776.20 was calculated (Kenyon et al., 2018). Based on their calculations, I believe the costs savings could be greater than that, as one unit of cell-salvaged blood is not equivalent to one unit of allogeneic, but possibly two to three units, thus representing an even bigger financial saving. Micro-costing of ICS in obstetrics in the UK has not been undertaken yet (to my knowledge) and would, therefore, be the obvious next step. Micro-costing analysis has been undertaken for allogeneic transfusion, estimating that it costs £57 for a unit of allogeneic red cell transfusion, and a further £36 per subsequent unit. This includes reagents, nursing time and consumables (Stokes et al., 2018). The cost of the unit (currently £128) has not been included, and so would take it to a total of approximately £185 per unit. Patient recovery and avoidance to potentially dangerous blood components also needs to be considered. I will shortly be undertaking a thorough UK-based cost-effective study of ICS compared to allogeneic transfusion.

CHAPTER 8 – NATIONAL AND INTERNATIONAL IMPACT

Locally, the work I have been involved in has been implemented into our Trust policy on intra-operative blood cell salvage for obstetrics (V1.3, Dec 2017). Our work has been presented at numerous national and European conferences – see page 70 for list.

One UK hospital referred to our work at a UK annual conference (Davies et al., 2018) and are now using our work as a baseline on how to set up a follow-up service. I will be working with them on undertaking a new project looking at fetal cell levels in maternal circulation, similar to what I have done previously, but on a much larger scale.

Our work was used to assist in writing the Cell Salvage Chapter in the latest SHOT annual report (Haynes & Ralph, 2018), although not directly referencing my work. And the UKCSAG guidelines/technical factsheets refer to our work. These are currently being reviewed and updated.

As our work is becoming more recognised, I have been asked by the Association for Perioperative Practice to write an updated article on obstetric cell salvage. This follows on from myself raising concerns about a report from 2011 republished in 2018 which contained outdated information.

With regards to my QC work, I am often asked for advice on ICS QC and have recently advised a large UK hospital on setting up a QC programme. Internationally I have had communication from as far as China regarding our QC work (Dong et al., 2014).

CHAPTER 9 – CONCLUSION

We were the authors to the first study to state that only one suction device is required in theatre, and also the first study to show it is possible to collect and potentially re-infuse blood lost from vaginal deliveries. We have shown it is possible to safely employ ICS routinely within an obstetric unit for caesarean sections with no additional staffing costs and a notable reduction in the rate of allogeneic blood transfusion. Intra-operative cell salvage is now established in all caesarean sections 24 hours a day, seven days a week with a dedicated machine within our obstetric theatre. Very few centres offer this. Intra-operative cell salvage is an economical and essential component of an obstetric patient management strategy.

Despite the limited follow-up data of test for antibody formation, data so far suggests that receiving a re-infusion of ICS processed blood does not put women at any more risk of antibody formation compared to women who receive an allogeneic transfusion. However, without more complete follow-up, rates may be deceptive, and as I have previously recommended, all Institutions using ICS in obstetrics should adopt this method of following up women three to four months post re-infusion in order to collect alloimmunisation rates and record this data on a central database.

Our work, and along with that of others, may be of great assistance in justifying the use of cell salvage in low-resource countries, where the allogeneic blood supply may not be as sufficient or as safe as of that in the UK.

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Poster presentation at the 9th Annual Network for Advancement of Transfusion Alternatives Annual Symposium 2008. Lisbon, Portugal.

Poster presentation at the annual meeting of the Obstetric Anaesthetists' Association 2009. Jersey, UK.

Poster presentation at the 11th Network for Advancement of Transfusion Alternatives Annual Symposium 2010. Barcelona, Spain.

Poster presentation at Annual Scientific Meeting of the British Blood Transfusion Society 2011. Glasgow, UK.

Poster presentation at Annual Scientific Meeting of the British Blood Transfusion Society 2011. Glasgow, UK.

Poster presentation at the 13th Annual Network for the Advancement of Transfusion Alternatives Annual Symposium 2012. Copenhagen, Denmark.

Poster presentation at the 13th Annual Network for the Advancement of Transfusion Alternatives Annual Symposium 2012. Copenhagen, Denmark.

Poster presentation at Annual Scientific Meeting of the British Blood Transfusion Society 2013. Birmingham, UK.

Poster presentation at Annual Scientific Meeting of the British Blood Transfusion Society 2013. Birmingham, UK.

Poster presentation at the 14th Annual Network for the Advancement of Patient Blood Management, Hemostasis and Thrombosis Annual Symposium 2013. Vienna, Austria.

Poster presentation at the 15th Annual Network for the Advancement of Patient Blood Management, Hemostasis and Thrombosis Annual Symposium 2014. Porto, Portugal.

Poster presentation at the 17th Annual Network for the Advancement of Patient Blood Management, Hemostasis and Thrombosis Annual Symposium 2016. Dublin, Ireland.

Poster presentation at the 17th Annual Network for the Advancement of Patient Blood Management, Hemostasis and Thrombosis Annual Symposium 2016. Dublin, Ireland.

Poster presentation at the 17th Annual Network for the Advancement of Patient Blood Management, Hemostasis and Thrombosis Annual Symposium 2016. Dublin, Ireland.

Poster presentation at the Annual Scientific Meeting of the British Blood Transfusion Society 2017. Glasgow, UK.

Poster presentation at the 19th Annual Network for the Advancement of Patient Blood Management, Hemostasis and Thrombosis Annual Symposium 2018. Lisbon, Portugal.

Poster presentation at the 19th Annual Network for the Advancement of Patient Blood Management, Hemostasis and Thrombosis Annual Symposium 2018. Lisbon, Portugal.

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Appendix 1

Sullivan, I., Faulds, J., & Ralph, C. (2008). Contamination of salvaged maternal blood by amniotic fluid and fetal red cells during elective caesarean section. *British Journal of Anaesthesia*, 101(2), 225-229.

Contribution by I Sullivan:

- Design including literature search
- Data collection
- Data analysis
- Manuscript writing

Number of citations:

Google Scholar:	81
Pubmed:	8
Web of Science:	40
Clarivate Analytics:	60

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OBSTETRICS

Contamination of salvaged maternal blood by amniotic fluid and fetal red cells during elective Caesarean section

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Background. Cell salvage in obstetrics is still a controversial subject and has yet to be fully embraced. The aim of this exploratory study was to measure amniotic fluid (AF), heparin, and fetal red cell contamination of washed filtered salvaged maternal blood and to investigate differences based on the number of suction devices used.

Methods. Patients undergoing elective Caesarean section were assigned alternately to one of two groups. In Group 1, all blood and AF was collected with one suction. In Group 2, AF was aspirated to waste with a second separate suction device before collection of any blood.

Results. In both groups, alpha-fetoprotein (AFP), squames cells, and heparin were significantly reduced ($P<0.001$) by the washing and filtering process. Mean AFP levels post-filtration were 2.58 IU ml^{-1} in Group 1 and 3.53 IU ml^{-1} in Group 2. Squames cells were completely removed in all but two cases. Fetal red blood cells were still present in the final product, range 0.13–4.35%. In Group 1, haemoglobin and haematocrit were higher than in Group 2, with lower white blood cell, AFP, and fetal red cell counts.

Conclusions. This study adds to the growing body of evidence that there is little or no possibility for AF contamination to enter the re-infusion system when used in conjunction with a leucodepletion filter.

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The use of cell salvage in obstetrics remains controversial. There is a concern of potential contamination of the salvaged blood with amniotic fluid (AF), which may cause AF embolus (anaphylactoid syndrome of pregnancy),[1] and the risk of alloimmunization of the mother with any fetal red blood cells aspirated.

Previous studies investigated the use of cell salvage in obstetrics and focused on the feasibility of the washing process in removing potential contaminants. After the use of a leucodepletion filter in cancer surgery, these filters were introduced into obstetric practice.[1] A later study using a newer advanced model demonstrated complete removal or a further reduction in contaminants by these filters.[2] Along with these results and later reports, the National Institute of Health and Clinical Excellence (NICE) published guidelines in 2005 suggesting that these filters can be considered for use in obstetrics.[3]

A common assumption was that two suction devices should be used, with as much AF aspirated to waste before any lost blood is collected.[1 4]

Although current evidence supports the use of cell salvage in obstetrics, a recent review suggested that more clinical data are required to add to this evidence and make the use of cell salvage more commonplace in obstetrics.[4]

The aim of the study was to add results to the current clinical evidence to support the use of cell salvage. We measured AF and fetal red cell contamination, and residual heparin levels of washed salvaged maternal blood when using either one or two suction devices, filtering the washed product through a leucodepletion filter for cell salvage (RS1 VAE PALL® Medical).

Methods

Information to perform power analysis and produce a well-designed prospective study is lacking due to the limited data available.[4] This study was set up as an exploratory study to investigate the safety and feasibility of cell salvage in obstetrics. Ethics Committee opinion was sought, but approval was not required. The sample size was dependent on the number of patients accessible over a set time period.

Written informed consent was obtained from 34 patients undergoing elective lower segment Caesarean section at the Royal Cornwall Hospital over a 4 month period. Patients were alternatively assigned to one of two groups before surgery. Group 1 (n=17) involved only one suction where all AF and blood was collected into the cell salvage machine, with Group 2 (n=17) using a second suction to aspirate as much AF to waste before any lost blood was collected.

All patients had a spinal anaesthetic apart from one who had an epidural top up; all spinals were performed using a Whitacre 25 gauge needle, with hyperbaric bupivacaine 0.5% and morphine 0.1 mg plus in some cases fentanyl 10–20 μg. In two cases, spinals had to be performed twice due to incomplete blocks and one was converted to a general anaesthetic.

During the surgical procedure, any blood lost was salvaged into a Dideco Electa Autotransfusion Cell Separator (Sorin Group, Milan, Italy). Aspirated blood was mixed with heparin 30 000 IU litre^{-1} of saline. Suction was set at 100–150 mm Hg with a prime at 100 ml min^{-1} and wash volumes of 900 ml on a continuing wash at 100 ml min^{-1}. The set was emptied at 150 ml min^{-1}. A 55 ml Latham bowl was used in all cases. In addition, any swabs from the surgical procedure were soaked in 1 litre of saline and 'compressed' at the end of case and solution aspirated, maximizing red cell collection. Processed washed blood was then gravity run through a Pall RS1 VAE leucodepletion filter (Pall Medical, Portsmouth, UK) into another re-infusion bag.

Pre-wash samples were taken from the collection reservoir, and post-wash and post-filtration samples were taken from the re-infusion packs, which were inverted gently several times to ensure even mixing before sampling.

The cell salvage machine use and sampling, in all cases, was performed by the same member of staff.

Pre-wash, post-wash, and post-filtration samples from both groups were tested for markers of AF contamination and heparin levels. Post-filtration samples were also tested to quantify any fetal red blood cells.

Six millilitres of blood were collected into a specimen tube and processed for alpha-fetoprotein (AFP) on an E170 Modular Analytics Analyser (Roche Diagnostics, Basel, Switzerland).

Four millilitres of blood were collected and centrifuged at 1600g for 5 min and processed for heparin levels on an Instrumentation Laboratory Futura Advance Coagulometer (Instrumentation Laboratory, MA, USA).

Six millilitres of blood were collected for fetal squames cell levels. After centrifugation at 1200g for 5 min, one part plasma was mixed with three parts Cytolyt Solution (Cytyc Corporation, West Sussex, UK). Vials were then centrifuged at 1200g for 5 min and supernatant discarded. Two to three drops of the cell bullet were then transferred to 20 ml PreservCyt Solution (Cytyc Corporation) and processed on a ThinPrep®2000 Processor (Cytyc Corporation) using blue-box non-gynae filters (Cytyc Corporation), generating one alcohol-fixed slide per sample. Slides were stained using the Papanicolaou technique. To avoid any potential carryover, pre-wash, post-wash, and post-filtration slides were stained separately using fresh reagents. Each slide was then examined under light microscopy for presence of fetal squames cells, with the whole slide being counted to exclude any variables, giving a result of number of cells per slide.

Two millilitres of blood were collected into EDTA for a full blood count to provide haemoglobin (Hb), haematocrit (HCT), and white blood cell (WBC) levels on a Bayer Advia 120 analyser (Bayer, Newbury, UK).

Post-filtration samples were also tested for fetal red blood cell quantification. Five millilitres of blood were collected into EDTA and processed with a monoclonal antibody to fetal Hb (IQProducts, Groningen, The Netherlands), with a Becton Dickinson FACSCalibur (NJ, USA) flow cytometer at 540 nm. Note: it was planed to perform all 34 cases for fetal red cells by flow cytometry, but due to unforeseen circumstances, the first seven cases were analysed using the Kleihauer–Betke technique.

Pre- and post-wash samples and post-wash and post-filtration samples were compared. Parametric data were analysed using a paired t-test, and non-parametric data analysed using a one-sample Wilcoxon test. $P<0.05$ was considered statistically significant. The observed power of the study was calculated after the collection of the data.

Results

Mean (SD) (range) age of patients in Group 1 was 32.1 (19–46) yr, and weight 82.7 (16.2) kg. In Group 2, the age was 32.1 (24–41) yr and body weight 82.4 (12.6) kg. Table 1 gives the reason for Caesarean section along with any coexisting diseases as shown in Table 2.

Table 1 Reasons for Caesarean section

Reason	Number
Previous section	23
Twins IVF	2
Back injury/pain	2
Breech	2
Failure to progress/Category 3	1
IUGR	1
Polyartetitisnodosa	1
Raised BP/pregnancy-induced hypertension	1
Placenta praevia	1

There were no surgical problems during the procedures. Four cases did not lose enough blood to obtain post-filtration samples (one from Group 1 and three from Group 2), with three further cases having partially filled bowls. A partial bowl was defined as a bowl that was not completely filled in the automatic mode of the cell salvage machine. These three bowls provided erroneous post-filtration results and, therefore, were excluded from analysis. The final blood product in these cases (one from Group 1 and two from Group 2) had a reduced Hb level of 7.1–10.3 g dl^{-1} and HCT of 21–31%, with a higher level of fetal red blood cells: 10.5% compared with 1.51%. Apart from fetal red blood cells in one case (Group 2), the values for Hb, HCT, and fetal red blood cells all were considerably above the upper 95% confidence limit for the full bowls.

The collected blood volume from Group 1 [1782 (354) ml] was slightly higher than that collected in Group 2 [1497 (562) ml], but the difference was not statistically significant (P=0.14). Similarly, on average, the red blood cell volume was higher in Group 1 [168 (77) ml] than in Group 2 [135 (94) ml], but again the difference was not statistically significant (P=0.30).

Table 3 shows the mean Hb, AFP, squames cells, and fetal red cell levels in the groups, along with statistical analysis.

Dependent on the manufacturer, the reference range for AFP is up to 10–15 IU ml^{-1}. The post-wash levels were significantly reduced (P<0.001) with no further reduction seen post-filtration (P=0.996). On average, there was a 98.7% reduction of pre-wash levels by the washing stage.

Mean heparin levels pre-wash were 1.33 IU ml^{-1} (95% CI 0.91–1.75 IU ml^{-1}) in Group 1 and 1.44 IU ml^{-1} (95% CI 0.82–2.05 IU ml^{-1}) in Group 2. Levels were reduced by 100% in both the groups (P<0.001) by the washing stage with no heparin being detected post-wash or post-filtration in all 34 cases.

Fetal squames cells were present in 12 out of 34 cases (six from each group) pre-wash, present in all post-wash samples, with a significant reduction post-filtration (P<0.001). Squames were present in two post-filtration samples, with two cells seen in each case. Both cases were from Group 1. Pre-wash samples were heavily diluted with AF and heparinized saline from surgery explaining why 22 samples were negative.

The mean percentage fetal red cells post-filtration from all cases (both groups) was 1.51% (95% CI 0.98–2.05), with a range of 0.13–4.35%. The first seven cases (three from Group 1 and four from Group 2) performed by the Kleihauer technique generated a mean 1.72% (95% CI −0.65 to 4.08), whereas the remaining cases performed by flow cytometry resulted in mean 1.47% (95% CI 0.92–2.01).

Table 2 Coexisting diseases

Co-morbidity	Number
Smoker	5
Diabetic	2
Asthma	2
Epilepsy	1
Protein S deficiency	1
Polyartetitisnodosa	1

Discussion

This study has shown the efficiency of the washing stage of the cell salvage machine, when used in combination with a leucodepletion filter, in significantly reducing levels of AF contaminants.

AFP was significantly reduced post-wash to levels well within the normal range for the general population, confirming the results from previous studies.[1 5 6]

During the study, the amount of heparin used was higher than with other surgical disciplines due to the hypercoagulable state of pregnancy. It would be expected that pre-wash samples were heavily contaminated with heparin, and this was confirmed during the study. It has been previously reported that residual levels of heparin remain in salvaged blood,[4] but we have now demonstrated the complete removal of heparin by the washing process in all 34 cases.

The role of fetal squames cells in AF embolus is still debated. In one study,[1] fetal squames cells were still present even with the additional step of filtering the washed blood through a leucodepletion filter, with only

Table 3 Analysis and comparison for Group 1 and Group 2. AFP, alpha-fetoprotein. Values are mean (95% CI). *We acknowledge that the observed power results are low; this, however, is one of the largest studies to date in the obstetric setting. †Mean HCT post-filtration was 45% in Group 1 and 42% in Group 2

	Group 1	Group 2	P-value	Observed power*
AFP pre-wash (IU ml^{-1})	253.72 (183.64–323.80)	381.60 (193.63–569.57)	0.19	0.26
AFP post-wash (IU ml^{-1})	2.71 (0.80–4.62)	3.14 (1.45–4.83)	0.72	0.06
AFP post-filtration (IU ml^{-1})	2.58 (0.54–4.62)	3.53 (1.29–5.76)	0.51	0.10
Hb pre-wash (g dl^{-1})	2.14 (1.52–2.75)	1.83 (1.17–2.50)	0.48	0.11
Hb post-wash (g dl^{-1})	16.95 (16.28–17.62)	16.20 (15.26–17.14)	0.18	0.27
Hb post-filtration† (g dl^{-1})	15.77 (15.07–16.47)	14.74 (13.70–15.78)	0.17	0.27
Squames cells pre-wash (cells per slide)	1.12 (−0.02 to 2.25)	0.94 (0.01–1.74)	0.80	0.06
Squames cells post-wash (cells per slide)	34.59 (10.89–58.28)	43.71 (14.42–73.00)	0.61	0.08
Squames cells post-filtration (cells per slide)	0.27 (−0.11 to 0.66)	0	0.18	0.26
Fetal red blood cells (%)	1.34 (0.56–2.12)	1.76 (0.95–2.57)	0.43	0.12

two out of 27 post-filtration cases being negative. Advanced filters may reduce this contamination.[2]

Of the 27 cases in our study, only two were positive post-filtration with two cells seen in each slide for each case. These were found when one suction was used. However, it is difficult to differentiate between fetal and adult squames cells. There is no evidence to show that fetal squames cells routinely enter the maternal circulation,[7] and so the significance of this contamination is difficult to quantify and in fact may have no clinical significance.

Fetal red blood cells were still present in the final product, consistent with previous studies,[2 6] and so could be significant in cases of red cell antigen incompatibilities between the mother and fetus. Rh(D) incompatibilities, although clinically significant, are generally avoided by routine prophylactic anti-D treatment throughout the pregnancy. However, fetal hyperbilirubinaemia and anaemia in future pregnancies can occur when antibodies have been formed to other red cell antigen incompatibilities. Examples of other clinically relevant antibodies that have been implicated in haemolytic disease of the newborn include anti-K, anti-c, anti-Fy(a), and anti-Jk(a).[8] Nevertheless, it must be appreciated that there is still a risk of alloimmunization of the mother either from transfusion of allogenic blood or a sensitizing event during pregnancy.

Current treatment of obstetric haemorrhage is with allogenic blood transfusion; however, there are concerns with allogenic blood. To date, there have been four confirmed cases of vCJD transmission via allogeneic blood, confirming that vCJD can be transmitted through blood transfusion.[9]

These potential risks of transfusion and reduction in availability of blood make it important to establish the use of cell salvage in obstetrics. It has been shown in more than 400 documented cases where cell salvaged blood has been returned to mothers with no significant adverse results.[4 10 11] Several key studies have commented on the use of cell salvage.[12–14]

This current study is one of the largest to date, with results that add to the growing body of evidence, showing there is little or no possibility for AF contamination to enter the re-infusion system, when used in conjunction with a leucodepletion filter. The role of the leucodepletion filter in this study has confirmed that it is required to remove AF contamination in cell salvage. WBC, platelets, and squames cells were still present post-wash but were significantly reduced by the filter. Whether the squames are of fetal or maternal origin is perhaps irrelevant as the filters have shown that they can remove both. As expected, AFP and heparin levels were not reduced by the filter, but heparin was completely removed in the washing process.

Regarding cell salvage in obstetrics, these results could be used to change the guidelines and allow routine re-transfusion of salvaged blood as opposed to overriding guidance in extreme emergency, and therefore allowing cell salvage to be used for elective and emergency cases. Even though it was not our intention to re-transfuse any salvaged blood in this study, ~500 ml was transfused to two cases. No changes in clinical state, heart rate, or clinical complications were noted, showing that in elective cases unexpected large blood loss can still occur and cell salvage can have a role to play in this situation.

This initial project acted as an exploratory study, and after the collection of these data, we have now implemented a comprehensive programme of cell salvage within our Trust to all women undergoing elective Caesarean sections, and further work is ongoing.

We conclude that one suction may be used in the obstetric setting, and washed filtered blood from partially filled bowls should not be re-transfused, regardless of the clinical situation. To obtain maximum washing efficiency in removing AF and fetal contaminants, only complete bowls in the automatic mode should be accepted.

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Appendix 2

Ralph, C., Faulds, F., & Sullivan, I. (2010). Cell salvage and leucocyte depletion filters. *Anaesthesia*, 65(12), 1228-1229.

Contribution by I Sullivan:

Article writing and editing

Number of citations:

Goggle Scholar:	2
Pubmed:	0
Web of Science:	0
Clarivate Analytics:	0

Thus, arguments based on financial loss are finely balanced.

'Needlestuck' staff become patients too, and staff who are denied the knowledge of patients' viral status are exposed to significant harms, as Hartle eloquently outlined. We are aware of several such cases in which doctors have come to significant harm as a result of probably unnecessary post exposure prophylaxis. Thus, the situation is not incapacitated patient vs professional staff member but patient vs patient, a situation that will be all too familiar to intensivists. The staff member's rights to autonomous informed consent, non-maleficence and justice are all ignored by current General Medical Council guidance (itself based on an untested interpretation of the relevant case law and statute).

We argue that in such cases, weighing the competing claims of the four principles leads to the moral conclusion that the incapacitated patient be tested. Burrows and Padkin have shown that the majority of our profession would probably draw the same conclusion. Furthermore, we argue that a reasonable court, be it criminal, civil or professional, would in all likelihood arrive at the same conclusion.

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Cell salvage and leucocyte depletion filters

We read with interest the recent editorial 'cell salvage induced hypotension and London buses' concerning hypotension associated with the use of the leucocyte depletion filters [1].

At the Royal Cornwall Hospital Trust, we routinely collect blood for cell salvage at the time of caesarean section. We are currently recruiting to a study involving re-infusion of cell-salvaged-blood and following up women post re-infusion. At the time of caesarean section, the collected blood is washed and processed if there is enough in the collection bowl, or if there are clinical signs of blood loss. We consent women for a re-infusion of the cell-saved blood. All women who have their blood returned are monitored during the re-infusion and are followed up at three and six months postoperatively. At follow-up, a blood sample is taken to test for antibody formation.

We have re-infused cell-saved blood to 70 patients. All these patients have received the re-infusion through a leucodepletion filter (Pall LeukoGuard® RS Filter; Pall Europe, Portsmouth, UK), and in all cases only one suction device was used. We have not observed any haemodynamic changes attributable to these re-infusions. However, we have not pressurised the bag or syringed the contents through the filter. We are aware that users have pressurised blood through the LeukoGuard RS filter without complication, although the manufacturer's product instructions state: 'use of this filter with a pressure cuff should comply with the recommendations of cell salvage equipment manufacturers' instructions for use'.

We have already demonstrated alpha-fetoprotein is significantly reduced post wash following cell-salvage, to levels well within the normal range for the general population, before passing through the filter. Heparin is also eliminated and whilst the washing process does not eliminate the presence of fetal squames, their significance in the circulation remains unknown [2].

Whilst the decision to re-infuse salvaged blood from a particular patient or patient group is a clinical one, the LeukoGuard RS filter has not been validated by the manufacturer for its ability to remove fetal squames from salvaged blood during re-infusion. Consequently, one cannot rely on the filter's ability to remove these contaminants during re-infusion of salvaged blood and the filter is not currently endorsed for use for this purpose. The product instructions state that the filter is only validated and indicated for the removal of leucocytes, fat particles and microaggregates from intra-operative salvaged-blood intended for re-infusion. We found the LeukoGuard RS filter significantly reduced the presence of fetal squames with only two out of 34 cases testing positive after filtration [2].

Transfusion-induced hypotension is a recognised phenomenon and has been reported both with and without the use of bedside leucocyte removal filters. Although some publications have suggested that this is an event secondary to the use of leucocyte removal filters [3], a 9-year study of adverse transfusion reactions found an overall incidence of anaphylactic or anaphylactoid reactions of 1.3% (21 of 1613) and of these, nine had associated hypotension [4]. None of these reactions involved the use of bedside leucocyte removal filters.

We recognise the limited flow achievable through a filter is a limitation when re-infusing cell-saved blood during hypovolaemic resuscitation. In such

cases, we estimate and provide minimal volume resuscitation and transfusion of peri-operative allogenic blood, and subsequently commence slower re-infusion of cell-saved blood. In patients who decline allogenic blood, such as Jehovah's Witnesses, we would consider removing the filter from the re-infusion.

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Fibrinogen concentrate use during major obstetric haemorrhage

Bell and colleagues described a series of six cases in which fibrinogen concentrate was used in combination with blood products to treat postpartum obstetric haemorrhage [1]. Other studies have also demonstrated a reduction in blood loss when using this blood product [2, 3]. Hypofibrinogenaemia is common in obstetric haemorrhage, especially if there is a consumptive coagulopathy [4] and we would like to report a case where fibrinogen concentrate was sufficient to arrest major antenatal vaginal bleeding after diagnosis of abruption and fetal death, before other blood products were available.

A 38-year-old multiparous woman was admitted at 34 + 2 weeks of gestation with a five-hour history of severe abdominal pain and no fetal movements. A transabdominal ultrasound scan revealed an intrauterine death, secondary to placental abruption, with a large retroplacental clot. On admission she had no vaginal bleeding, but was tachycardic with a pulse rate of 116 beats.min^{-1} and a blood pressure of 108/70 mmHg. We administered a bolus of 500 ml of colloid solution and commenced Hartmann's solution at 125 ml.h^{-1}, and sent blood for cross-matching. Initial blood results (taken at the time of admission, but available two hours later) showed haemoglobin (Hb) 8.1 g.dl^{-1}, platelets 258×10^9.l^{-1}, fibrinogen 1.9 g.l^{-1}, activated partial thromboplastin time (APPT) 27 s and prothrombin time (PT) 14.5 s.

As the blood results became available, she had a sudden vaginal blood loss of 750 ml followed by continuous bleeding of 200–300 ml every 15 min; her blood pressure was 89/56 and heart rate 114 beats.min^{-1}. She looked pale and peripherally shut down. We took a further full blood count and coagulation test, but based on the clinical deterioration, the previous low fibrinogen of 1.9, the history of abruption and the likelihood of fulminating disseminated intravascular coagulopathy (DIC), a consultant haematologist advised that 4 g fibrinogen concentrate should immediately be given with four units of fresh-frozen plasma.

We administered the fibrinogen concentrate (CSL Behring, King of Prussia, PA, USA) within 15 min, with two units of blood, and within 10 min her vaginal bleeding stopped. We then administered the fresh frozen plasma and a further two units of blood. Her clinical picture improved over the next 30 min; she had artificial rupture of membranes and labour was induced with an oxytocin infusion at 0.06 units.h^{-1}. The blood results taken before and after the blood products showed Hb 6.8 g.dl^{-1}, platelets 241×10^9.l^{-1}, fibrinogen 0.9 g.dl^{-1}, APPT 34.6 s and PT 15.8, and Hb 6.1 g.dl^{-1}, platelets 111×10^9.l^{-1}, fibrinogen 1.5 g.dl^{-1}, APPT 36.5 s and PT 15.7 s, respectively.

We gave her further three units of blood and four of fresh frozen plasma, on the basis of a third set of blood results. There was no further bleeding, her clinical condition stabilised and she had a spontaneous vaginal delivery of a dead fetus with a 2000-ml blood clot three and-a-half hours later. At delivery, she was normotensive, her heart rate had settled to 90 beats.min^{-1}, she was passing over 100 ml.h^{-1} of urine and she was peripherally well perfused. Following delivery, she received no further blood products although her platelets fell to 57×10^9.l^{-1} and she had three 500-mg doses of tranexamic acid on the advice of the haematologist.

We believe that this is the first report of major antenatal obstetric haemorrhage associated with DIC arrested by fibrinogen concentrate alone, which allowed induction of labour and subsequent vaginal delivery. Fibrinogen concentrate was available more quickly than other clotting products as it is rapidly solubilised from an ampoule in 50 ml water and given as a bolus. To raise the plasma fibrinogen concentration by 1 g.l^{-1} in a 70-kg person, 1000 ml fresh frozen plasma (6 standard UK units), or 260 ml cryoprecipitate (10 standard UK units) will be required [5]. Administration of adequate doses of fresh frozen plasma or cryoprecipitate to treat hypofibrinogenaemia during obstetric haemorrhage will therefore take a substantial amount of time, even with an efficient blood bank and portering system. This case also highlights the importance of monitoring the fibrinogen levels in obstetric haemorrhage and treating hypofibrinogenaemia early and aggressively as the APTT, PT and platelet count were unreliable during the initial presentation.

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Appendix 3

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Contribution by I Sullivan:

Design including literature search

Data collection

Data analysis

Manuscript writing

Number of citations:

Google Scholar:	24
Pubmed:	2
Web of Science:	9
Clarivate Analytics:	6

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BJA

OBSTETRICS

Intraoperative cell salvaged blood as part of a blood conservation strategy in Caesarean section: is fetal red cell contamination important?†

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Editor's key points

- Intraoperative cell salvage (IOCS) is increasingly used as part of a blood conservation strategy.
- Its widespread use in obstetrics has been hindered by concerns over amniotic fluid embolism and fetal red cell contamination.
- In this series of 70 patients, the incidence of red cell contamination resulting in antibody formation was very low.
- IOCS reduced the requirements for allogeneic blood.

Background. Cell salvage is used in obstetric surgery as part of a blood conservation strategy in our Trust. This carries a theoretical risk of amniotic fluid embolism and also a risk of fetal red cells being present in the re-infusion, resulting in alloimmunization. In this study, we attempted to quantify the risk of antibody formation from re-infusion of autologous blood after Caesarean section.

Methods. Women presenting for elective Caesarean section were routinely requested to consent for collection of blood by cell salvage, using one suction device. If an adequate volume of blood was collected, it was processed and, if clinically appropriate, re-infused via a leucodepletion filter. Women who received a re-infusion were followed up to test for antibody formation.

Results. Seventy women consented for re-infusion and follow-up. The median volume re-infused was 324 ml (range 118–1690 ml). The median fetal red cell contamination was 0.8 ml (range 0.2–12.9 ml). All re-infusions were given without adverse clinical signs. No antibodies were detected in 48 follow-up samples. One positive anti-S antibody was detected.

Conclusions. The implementation of a blood conservation strategy which includes the use of intraoperative cell salvage appears safe and can contribute to a reduction in the number of blood transfusions to the obstetric population. We remain uncertain of the significance of fetal red cell contamination.

Keywords: amniotic fluid embolism; blood transfusion; blood transfusion, autologous; Caesarean section

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Allogeneic blood transfusion carries potentially catastrophic risks of infection and incompatibility reactions. To conserve allogeneic blood, we revised our local transfusion guidelines, introduced i.v. iron therapy where indicated, and established intraoperative cell salvage (IOCS) into routine practice in our obstetric operating theatre.

Despite the benefits of transfusing autologous blood, there remains a perceived risk of contamination with amniotic fluid, a risk of alloimmunization of the mother by fetal red blood cells (RBCs), and a belief that the introduction of IOCS will require additional resources.

We concluded from earlier work that there was little possibility for amniotic fluid contamination to enter the re-infusion system when used with a leucodepletion filter and that cell salvaged blood was safe.[1] However, we were unable to quantify the significance of fetal red cell contamination or red cell antigen incompatibilities between the mother and fetus.

The aim of this study was to quantify the fetal red cell contamination of maternal salvaged blood and assess the risk of this contamination. The secondary aim was to add to the growing evidence that supports the safe use of IOCS in obstetrics.

Methods

All women undergoing elective Caesarean section at the Royal Cornwall Hospital Trust were asked to participate in the study. They were informed at the Pre Assessment Clinic of the benefits and risks of cell salvage and received a patient information sheet, and surgical consent was obtained for the use of cell salvage. Women having Category

†Presented in part at the Obstetric Anaesthetists' Association meeting, Jersey, 2009.

2 or 3 Caesarean sections, who were able to read the patient information sheet before the re-infusion of autologous blood, were also asked to participate in the study. Category 1 emergency sections were excluded as were all women who received cell salvaged blood without a chance to read the patient information leaflet. Ethical committee approval was gained from Cornwall and Plymouth Research Ethics Committee to study the contamination of Salvaged Maternal Blood by Fetal red Cells during Caesarean section (ref: 07/H0203/257).[1]

During surgery, any blood lost (blood with amniotic fluid) was collected with a specific wide-bore sucker into a reservoir in a Haemonetics Cell Saver® 5 system (Haemonetics Corp., Braintree, MA, USA). The suction line was anticoagulated (30 000 IU heparin per litre of saline), pre-prepared, and attached to power of 150 mm Hg. Suction pressure was increased to a maximum of 300 mm Hg in the presence of heavy bleeding, although higher settings were avoided if possible as these can damage red cell structure. Although a Yankauer tip can be attached, it was not used routinely because it can theoretically compromise red cell integrity.[2]

The surgeon and assistant aimed to maximize blood collection via the wide-bore sucker as red cell retrieval was more effective from aspirated blood than blood extracted from washed swabs. However, when the swabs were heavily soiled with blood, they were washed and blood collected from the wash. Fetal blood spillage was kept to a minimum whenever possible, with the surgeons cutting the umbilical cord close to the clamp.

If the surgeon or anaesthetist judged the blood loss to potentially require replacement and there was sufficient blood aspirated into the reservoir, then the collection was processed. The final decision to re-infuse was made jointly by the obstetrician and anaesthetist in discussion with the woman whenever possible, and this discussion was documented. The salvaged blood bag was labelled with a Patient Identification Label which corresponded to the patient's wristband and was prescribed in the usual way on the i.v. fluids prescription chart. Before a decision to use the blood, the bag remained at the patient's bedside and was not stored in a fridge. If the blood was to be re-infused, a patient blood sample was obtained for test of fetal red cell contamination, before re-infusion.

Re-infusion of the salvaged blood usually took place within 2 h of collection but had to occur within 6 h of the start of the collection. Written informed consent was obtained from all women who received a re-infusion. This consent was usually obtained after delivery, but when significant blood loss was predicted, the consent was taken before surgery. All women received the processed washed blood through a leucodepletion filter (Pall LeukoGuard® RS Filter, Pall Europe, Europa House, Portsmouth, UK). Blood warmers and pressurized bags were not used. Routine observations were continued throughout the re-infusion.

A monitoring sheet was completed for all cases, even if collection only (no processing or re-infusion) occurred (see Supplementary Appendix 1).

A blood sample was obtained from the post-filtration re-infusion bag. Initially, the protocol required no further sampling direct from the patient; however, an amendment to the original protocol (January 2010) included a patient sample to be obtained before re-infusion. Samples were sent directly for processing to test for full blood count and using the Kleihauer–Betke technique to detect fetal red cells.

Consenting patients agreed to return for a follow-up appointment 3–6 months after surgery, at which time, a sample of venous blood was obtained to screen for any maternal antibodies. Any positive antibody screens were investigated further, discussed with the Consultant Haematologist and Consultant Obstetrician, the significance assessed, and, where appropriate, communication with the patient was undertaken by a consultant and the Ethics Committee informed.

Results

Seventy women were enrolled to the study, with mean age 33 yr (range 22–43 yr). All were ASA class I or II. Forty-eight of the 70 women recruited were undergoing elective surgery (Category 4 Caesarean section). Nineteen were having Category 3 or 2 sections, eight of whom had some bleeding before operation. The remaining three cases were women who were taken to the operating theatre because of bleeding.

The mean preoperative haemoglobin (Hb) concentration was 11.3 g dl^{-1} [SD 1.0 (range 8.7–13.3 g dl^{-1})]. These higher concentrations reflect that the majority of cases were undergoing elective operations and not bleeding before surgery. The median estimated blood loss during the procedure was 900 ml (range 400–7000 ml). Collected blood was only processed if there was an estimated blood loss of more than 1 litre and only re-infused if a full bowl was processed.

When possible, before re-infusion, postoperative Hb concentrations were measured either with a HemoCue (HemoCue AB, Angelholm, Sweden) device at the bedside or a formal laboratory sample. The mean Hb concentration after operation was 9.0 g dl^{-1} [SD 1.3 (range 6.6–11.5 g dl^{-1})].

Re-infusions were offered to all women, regardless of the postoperative Hb concentration. With informed consent, some women chose to have the re-infusion of autologous blood, despite normal Hb concentrations.

The median total collected and processed volume was 2339 ml (range 1458–6723 ml). This resulted in a median RBC re-infusion volume of 324 ml (range 118–1690 ml).

Thirteen of the 70 women who received autologous blood also had allogeneic blood ordered and issued, and went on to receive one or more units of blood in addition to the re-infusion, ranging from one to 16 units. Four women received other blood products (fresh frozen plasma, cryoprecipitate, and platelets) in addition. Two of the four women who received additional blood products were bleeding before operation and had a ruptured uterus. The other two women were not bleeding before operation; one had an elective section for a fibroid and the other a Category 3 section for placenta praevia/accreta.

Twelve of the 13 women received their allogeneic blood transfusion in the delivery suite and one in the post-natal ward.

Samples obtained from the processed re-infusion packs were collected into EDTA and processed on a Bayer Advia 120 Full Blood Count analyser (Bayer, Newbury, UK), providing Hb and haematocrit (Hct). Samples were also tested to quantify any fetal RBCs present, using the Kleihauer–Betke technique.

The mean Hb concentration of the processed blood was 14.2 g dl^{-1} [SD 2.0 (range 10.8–18.9 g dl^{-1})] with a mean Hct of 0.418 [0.06 (range 0.322–0.541)].

In all samples obtained from the processed re-infusion packs, fetal RBCs were detected, with a median contamination volume of 0.8 ml (range 0.2–12.9 ml). Two cases were excluded from calculations due to being significant outliers with contamination of more than 25 ml. There were potentially user errors in the sampling method, resulting in poor quality of the Kleihauer–Betke slides.

All women consented for re-infusion consented for follow-up and were contacted between 3 and 6 months for a follow-up appointment. Eighteen women were uncontactable. Forty-eight women attended follow-up and had samples tested for antibody formation. One positive antibody was detected. Investigations confirmed this as anti-S. The clinical significance of this antibody in obstetrics is unknown. The remaining four cases are due for a follow-up appointment in the next few months.

As part of the blood conservation programme, figures for the consumption of blood throughout the Trust were also compared with those used in obstetrics. Data from the previous 3 yr demonstrated a reduction in blood transfusion in the obstetric population.

Fifty-eight patients (in 2007/08) needed an allogeneic blood transfusion during or immediately after delivery. Sixty-two were transfused in 2008/09 and 40 in 2009/10. The total number of units transfused decreased from 244 (2007/08) to 241 (2008/09) to 197 (2009/10), with the percentage of overall transfusions used in obstetric practice decreasing from 2.3% to 1.7%. The birth rates in each year were similar. The use of IOCS increased during the study period and was greatest during 2009/10, which may in part account for the greater reduction in the figures for this period.

Discussion

Cell salvage and autologous blood transfusion is an established method of blood conservation for surgical procedures that are anticipated to involve significant blood loss. However, despite the endorsement of the AAGBI, OAA, and NICE, its use is not established in obstetric practice.[3 4] We found that the introduction of IOCS has been associated with a reduction in allogeneic blood transfusion in obstetric practice over the past 3 yr. This has been demonstrated in other units and speciality areas, and we were keen to establish cell salvage as part of our blood conservation strategy in obstetrics, where surgical cases can cause unpredictable and significant blood loss.[5 6] The risks of allogeneic blood are significant, leading to a review of the triggers for transfusion and implementation of other strategies to conserve blood.[7 8] For the 70 women having IOCS in this study, the availability of autologous blood resulted in an over-estimation of the need for allogeneic blood, with blood ordered being either all or partly returned unused (14 and 13 women, respectively).

One reason for the slow adoption of IOCS in obstetrics has been the perceived risk of amniotic fluid embolism. In earlier work, we demonstrated α-fetoprotein is significantly reduced post-wash after cell-salvage, to concentrations well within the normal range for the general population, before passing through the filter. Heparin is also eliminated, and while the washing process does not eliminate the presence of squame cells, their significance in the circulation remains unknown.[1] Squame cells present post-wash are significantly reduced post-filtration. It is therefore recommended to re-infuse through a leucodepletion filter which removes any remaining fetal contaminant. As there is little or no possibility of amniotic fluid contamination re-entering the re-infusion system and, of the 1.1% of reports to SHOT associated with autologous transfusion reactions, there have been no reports of death or major morbidity indicative of a sudden obstetric collapse, this risk remains entirely theoretical.[7]

Other perceived barriers to implementing IOCS are costs, manpower planning, and training. We have overcome these issues by establishing the cell saver into routine practice. In our unit, the set-up and use of IOCS is included into the routine preparation of the obstetric operating theatre and is entirely run by the normal quota of obstetric theatre staff. This has been aided by the introduction of a competency training programme for operating department practitioners (ODP) and we use one ODP to both assist the anaesthetist and operate the cell saver machine. No additional manpower has been required to facilitate the use of cell salvage and it is currently used for more than 40% of all our Caesarean sections. We only use one suction device, as the washing process is effective in removing α-fetoprotein, and contamination is similar using one or two suction devices; using one suction device reduces waste and improves blood collection volumes.[1] The suction line is pre-prepared, enabling theatre personnel to commence IOCS, even in urgent or emergency cases. Over the last 2 yr, blood has been collected but not processed in 450 patients. Thus, costs have been minimized by processing only those collections where significant blood loss occurs and allogeneic transfusion is likely. All re-infusions have been administered through a leucodepletion filter by gravity alone. No haemodynamic changes were observed during the re-infusions, although recent case reports have reported hypotension after rapid re-infusions through a leucodepletion filter.[9] Transfusion-induced hypotension is a recognized phenomenon and has been reported both with and without the use of bedside leucocyte removal

filters.[10 11] In cases requiring rapid resuscitation, we use volume resuscitation with little or no perioperative transfusion of allogeneic blood, allowing the subsequent administration of the slower re-infusion of cell salvaged blood. In patients who decline allogeneic blood, such as Jehovah's Witnesses, we would consider removing the filter from the re-infusion.[12 13]

There remains a risk of alloimmunization of the mother by re-infusion of fetal RBCs in the final washed product. These may be significant in cases of red cell antigen incompatibility between the mother and fetus. However, the potential for sensitization can occur throughout pregnancy and at delivery, as concentrations of fetal RBCs in the maternal circulation increase during pregnancy. Peak transplacental haemorrhage (TPH) occurs at delivery, with nearly 1% of women in the third trimester having TPHs of >2.5 ml and 0.3% having >15 ml.[14] We previously reported similar amounts of contamination in parturients with a median fetal RBC volume of 0.48 ml (range 0–4.6 ml) before delivery and a maximum of 9 ml after delivery, although many of these women had an obvious TPH during delivery.[15] Therefore, re-infusing a median fetal red cell volume of 0.8 ml (range 0.2–12.9 ml) in cell salvaged blood is comparable with that found normally in the maternal circulation after delivery. The maximum we have re-infused has been 12.9 ml, and in two cases (excluded from the analysis), contamination volumes were >25 ml. Both these re-infusions (320 and 280 ml) were to women who delivered twins. Other reported volumes of fetal RBCs collected by cell salvage range from 0.2 to 19 ml.[16–18] The critical volume of contamination required to provoke an antibody response or an immune response to red cell antigens (Kell, Duffy, and Rh, for example) is unknown.

Data from a 3 yr period show that 0.4% of obstetric patients at our Trust have a clinically significant antibody. Some of these antibodies resulted from pregnancy or, fewer, after an allogeneic blood transfusion (Table 1). The formation of anti-D in mothers of an Rh(D)-positive fetus, although clinically significant, has been reduced by the use of routine prophylactic anti-D treatment throughout the pregnancy. Less commonly, other clinically significant antibodies, such as anti-K, anti-c, anti-Fy(a), and anti-Jk(a), have been implicated in haemolytic disease of the newborn.[19]

Follow-up of 48 cases has detected one case of anti-S antibodies. This was not detected antenatally and the clinical significance of this is unknown. We were unable to establish whether the anti-S was produced due to the cell salvage re-infusion or due to contamination from multiple antenatal bleeds suffered by this patient from 33 weeks gestation or from the significant placental abruption requiring delivery by Caesarean section. Estimated blood loss at the time of operation was 2.5 litre; 400 ml autologous blood was re-infused. We feel it was more likely that the antibody anti-S was produced due to the multiple TPH during pregnancy and delivery, although we cannot exclude cell salvage with certainty.

Table 1 Origin of antibodies detected

	Unknown cause	Due to pregnancy	Due to allogeneic blood transfusion
Formation of antibody (%)			
2007/08 (*n*=26)	65	31	4
2008/09 (*n*=25)	40	40	20
2009/10 (*n*=23)	43	48	9

Owing to the low incidence of antibody formation and relatively small numbers of patients followed up, it is impossible to speculate if the incidence of antibody formation or alloimmunization after autologous blood transfusion is greater, less, or the same as that which occurs in pregnancy. Despite this, there already has been a suggestion that the risk of alloimmunization is unlikely to be greater than that incurred in a normal vaginal delivery.[20]

Large numbers of women need to be followed up to assess the incidence of antibody formation and also to evaluate the introduction of cell salvage into routine practice.[21] We suggest that a central database is formed to collate this information and assess the risk of alloimmunization.

In conclusion, the introduction of cell salvage in obstetric surgery can reduce allogeneic blood transfusions. The procedure is efficient, and IOCS has been introduced with minimal manpower or resource implications. The significance of the risk of alloimmunization by fetal RBCs is uncertain after a re-infusion of cell salvaged blood. Further work and collaboration is required to assess this risk and we recommend that all women are followed up to test for antibody formation 3–6 months after re-infusion.

Supplementary material

Supplementary material is available at *British Journal of Anaesthesia* online.

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Conflict of interest

None declared.

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Appendix 4

Sullivan, I.J., Hicks, M.K., Faulds, J.N., Carson, P.J., & Noble, R.S. (2012). A modified thrombin clotting time test as a quality control marker for heparin contamination in obstetric intraoperative cell salvage. *Transfusion Medicine*, 22(1), 68-70.

Contribution by I Sullivan:

Design including literature search

All laboratory analysis

Manuscript writing

Number of citations:

Google Scholar:	3
Pubmed:	1
Web of Science:	1
Clarivate Analytics:	1

TRANSFUSION MEDICINE Official Journal of the British Blood Transfusion Society

Transfusion Medicine | SHORT COMMUNICATION

A modified thrombin clotting time test as a quality control marker for heparin contamination in obstetric intraoperative cell salvage

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SUMMARY

Objective: To assess if a modified thrombin clotting time test could be used as a simple quality control (QC) method to screen for unfractionated heparin in the product obtained from obstetric intraoperative cell salvage cases before re-infusion.

Background: A national QC scheme has recently been piloted to monitor the quality of autologous blood being returned to the patient. Laboratory tests include full blood count and microalbumin. Unfractionated heparin testing should be performed to ensure that there is no gross contamination of heparin in the final product; however, presently, there is no quick cheap test available suitable for heparin detection.

Materials and Methods: Samples were collected into plain non-anticoagulated tubes and centrifuged at 2500 × *g* for 5 min. Supernatant was mixed with commercially available coagulated normal plasma and a thrombin clotting time test performed.

Results: Calibration runs demonstrated that our system was sensitive up to 0·14 IU mL^{-1} heparin, linear between 0·08 and 0·14 IU mL^{-1}.

Conclusion: We have shown that the thrombin clotting time test can be modified and used as a cheap and reliable marker for heparin contamination. We have successfully incorporated this modified test into our hospital's obstetric QC scheme.

Key words: cell salvage, intraoperative, obstetrics, quality control.

The use of intraoperative cell salvage (ICS) and autologous blood transfusion has become an important method of blood conservation, with the main aim of reducing allogenic transfusions and its associated complications.

Spilled blood is aspirated from the operation site through a double lumen suction device into a collection reservoir. The salvaged blood is mixed with heparinised saline at the point of suction/collection with a pre-prepared volume of heparinised saline. The red blood cells undergo a washing and centrifugation process and are re-suspended in normal saline ready for re-infusion. This process is known to remove, or reduce, the levels of free haemoglobin, clotting factors, platelets, white blood cells and heparin (Catling & Thomas, 2006). Previous studies have shown that heparin was still present in the product, although not at levels to inhibit clotting (Umlas & O'Neill, 1981; Kling *et al.*, 1988; Burman *et al.*, 2002). We have recently demonstrated that complete removal of heparin is possible in 34 of 34 samples (Sullivan *et al.*, 2008).

A national quality control (QC) scheme has recently been piloted in the UK to monitor the quality of autologous blood being returned to the patient (Faulds, 2009). Laboratory tests include a full blood count (haemoglobin/haematocrit) to assess the quality of the end product and microalbumin as a marker of the washing efficiency. It was deemed important that, if possible, heparin testing is performed to ensure there is no gross contamination of heparin in the final product being returned to the patient. There have been concerns from surgeons that heparin may still be present in final end product which could compromise the patient's recovery.

There is no quick cheap test available suitable to screen for heparin contamination. The activated partial thromboplastin time is used to monitor heparin anticoagulant therapy but cannot reliably screen for heparin at low levels. The anti-Xa assay is expensive and may not be available in all the laboratories. The thrombin clotting time test was deemed as a suitable alternative due to its sensitivity to heparin, which results in prolongation of the test when heparin is present. Reconstituting thrombin reagent with imidazole-buffered saline, as opposed to calcium chloride, will also increase the sensitivity further (Carr *et al.*, 1986).

The aim of this investigation was to assess if the routine laboratory thrombin clotting time test could be modified and

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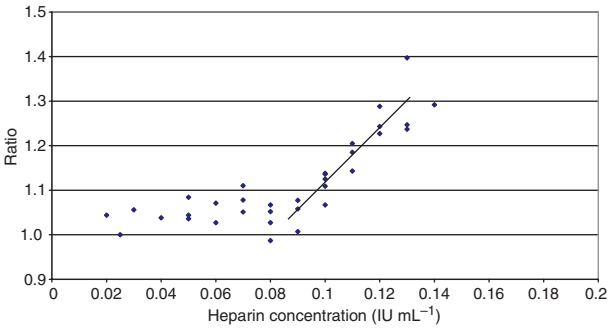

Fig. 1. Calibration curve for intraoperative cell salvage samples processed by the modified thrombin clotting time test.

used as a simple marker to detect gross heparin contamination in the product of ICS before re-infusion.

MATERIALS AND METHODS

A sample was collected from the re-infusion pack into plain non-anticoagulated blood bottles and centrifuged at 2500 × *g* for 5 min. Supernatant from the cell salvage sample was mixed 1 : 1 with commercially available coagulated normal plasma and the thrombin clotting time test performed. Normal plasma was used as a standardised source of fibrinogen.

Samples were processed on an ACL TOP analyser (Instrumentation Laboratory UK Ltd, Cheshire, UK) using the pre-set test. Eighty microlitres of supernatant from cell salvage sample was added to 80 μL of Instrumentation Laboratory (Instrumentation Laboratory UK Ltd) normal plasma. Instrumentation Laboratory thrombin reagent (concentration 3·0 UNIH mL^{-1}) was reconstituted with imidazole-buffered saline (pH 7·4). Hundred microlitres of this reagent was added to the above and time to clot was measured. Acquisition time for the test was set at 300 s.

With each batch, a 'blank' was run. This consisted of imidazole buffer being substituted with the cell salvage sample supernatant, added to normal plasma and thrombin clotting time test was performed. This allowed clotting times to be converted to a ratio. The thrombin reagent was adjusted to give a normal clotting time of 15 s.

As part of initial investigations, four calibration runs were performed on separate occasions, showing reproducibility and consistency. Buffer samples spiked with known amounts of heparin were tested and the calibration curve was plotted. Ratios obtained from cell salvage samples could then be read off the calibration curve to ascertain approximate heparin concentration.

Allowing for reagents, rinse, cuvettes and buffer equates to approximately £0·70 per test, compared to approximately £4·00 per test using the recognised anti-Xa assay on the analyser.

RESULTS

Figure 1 shows the calibration curve.

DISCUSSION

We have shown that the thrombin clotting time test can be modified and used as a simple marker for heparin contamination in obstetric cell salvage when unfractionated heparin is used as the anticoagulant.

This modified thrombin clotting test was shown to be a reliable and sensitive marker in measurement of heparin between 0 and 0·14 IU mL^{-1} heparin, and linear between 0·08 and 0·14 IU mL^{-1} (cv 0·002). Concentrations >0·15 IU mL^{-1} heparin resulted in no measurable clot being detected on the analyser. It has not been validated for acid citrate dextrose (ACD) and further investigations on whether this test can be used when ACD is the anticoagulant will be performed.

The test was designed to screen to exclude gross heparin contamination. This technique can only detect up to 0·15 IU mL^{-1} heparin, which equates to a clinically insignificant amount that could be safely re-infused. Only at levels >0·5 IU mL^{-1} would heparin significantly affect *in vivo* coagulation.

During a quality assurance pilot programme (Faulds, 2009), it became apparent that no routinely available test could provide a rapid measure of clinically significant heparin contamination. The anti-Xa assay is expensive and not readily available. As a pre re-infusion screen, our method is readily available and reduces those specimens that might require a factor Xa assay in QC of ICS.

Initial calibration and testing of cell salvage samples were performed on obstetric cases. It was extended to include other surgical specialities. In orthopaedic cases the assay is unreliable, possibly owing to a haemolysed end product. Manufacturer's product information for the thrombin time reagent indicate that free haemoglobin must exceed 0·5 g dL^{-1} before it interferes with the test. The degree and significance of haemolysis in orthopaedic cell salvage require a separate study.

We conclude that this modified test is a cheap (<£1 per test), quick and reliable marker for heparin contamination in obstetric cases where cell salvage is used. Further testing when using cell salvage for vascular, urology and gynaecology cases is planned. We have successfully incorporated this modified test into our hospital's QC scheme for ICS.

ACKNOWLEDGMENTS

I. J. S. performed the research, analysed the data and wrote the paper. M. K. H. helped to design and perform initial research. J. N. F. provided essential reagents and advice on the cell salvage QC programme, and helped to write the paper. P. J. C. helped to design the technique and analyse the data, and reviewed the paper. Dr R. S. N. reviewed the paper.

CONFLICT OF INTEREST

The authors have no competing interests.

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Appendix 5

Sullivan, I.J., & Faulds, J.N. (2013). Lactate dehydrogenase and haemolysis index as quality control markers of haemolysis in intra-operative cell salvage. *Transfusion Medicine*, 23(5):326-329.

Contribution by I Sullivan:

Design including literature search

All laboratory analysis

Manuscript writing

Number of citations:

Google Scholar:	10
Pubmed:	2
Web of Science:	4
Clarivate Analytics:	3

TRANSFUSION MEDICINE Official Journal of the British Blood Transfusion Society

Transfusion Medicine | ORIGINAL ARTICLE

Lactate dehydrogenase and Haemolysis Index as quality control markers of haemolysis in intra-operative cell salvage

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Received 24 March 2013; accepted for publication 18 June 2013

SUMMARY

Objectives: The aim of this investigation was to explore the potential use of the tests lactate dehydrogenase (LDH) and Haemolysis Index as haemolysis markers in intra-operative cell salvage (ICS) blood in comparison to plasma free haemoglobin levels.

Background: Quality control (QC) should be seen as a fundamental part of any ICS blood conservation programme, however, due to lack of available knowledge, familiarity and experience, QC is still a comparatively new subject. A QC pilot scheme is currently being undertaken by the Royal Cornwall Hospital in association with the UK Cell Salvage Action Group to explore potential markers that can be used to assess the quality of blood obtained from ICS. This test list should be available to all ICS users and achievable within financial budgets. Currently this proposed test list includes a full blood count, a protein marker such as urine albumin/microalbumin and heparin monitoring. Haemolysis testing is another key marker.

Methods/Materials: Samples were collected from ICS processed blood and allogeneic SAGM leucodepleted red cell units and processed for plasma free haemoglobin, LDH and Haemolysis Index.

Results: There was a very strong correlation between plasma free haemoglobin and LDH (0·960), and plasma free haemoglobin and the Haemolysis Index (0·944).

Conclusion: We have shown that the LDH and Haemolysis Index tests are suitable and reliable alternatives for measuring haemolysis from samples obtained from ICS or allogeneic blood. We have incorporated the LDH test into our Hospital's ICS QC package and recommend that this test is considered for all ICS QC samples.

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Key words: allogeneic blood, free haemoglobin, haemolysis, intra-operative cell salvage, lactate dehydrogenase, quality control.

Quality control (QC) is an integral part of laboratories though less common in clinical anaesthesia (Kelleher *et al.*, 2011). QC should be seen as a fundamental part of any intra-operative cell salvage (ICS) blood conservation programme, however, due to lack of available knowledge, familiarity and experience QC is still a relatively new subject. A recent report showed that '58% of respondents do not currently QC ICS machines and 68% do not QC the operators of ICS' (Jones & Howell, 2011). What few centres do undertake QC employ their own control measures, with no standardised process. It is hoped that a benchmarking process for QC can be developed allowing centres to compare processes and performances.

A pilot scheme is currently being undertaken by the Royal Cornwall Hospital in association with the UK Cell Salvage Action Group to explore potential markers that can be used to assess the quality of blood obtained from ICS. This test list should be available to most hospital users, while achievable within tightening financial budgets. Presently this proposed test list includes a full blood count (haemoglobin and haematocrit), a protein marker such as urine albumin/microalbumin, heparin monitoring and additionally being able to quantify haemolysis.

Haemolysis is the breakdown of red cells and results in free haemoglobin being released into circulation. Within ICS this can be caused by the surgical procedure as well as occurring at the collection (aspiration method, vacuum pressures for example) or processing stages. It has been suggested that haemolysis levels may increase during machine use (Hansen *et al.*, 2004).

Plasma free haemoglobin, as a measure of haemolysis, can be quantified using spectrophotometry or enzyme-linked immunosorbent assay (ELISA) techniques. It is recommended that routine haematology laboratory analysers are not used to measure plasma free haemoglobin due to the reduced sensitivity at the lower ranges. For example, one type of full blood count (FBC) analyser has a reported lower detection range of 0·0 g dL^{-1}

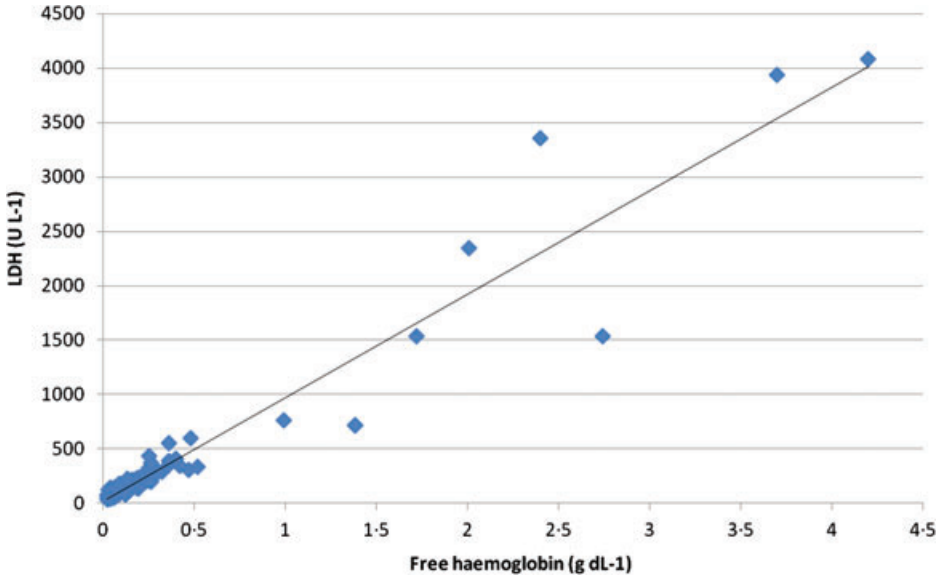

Fig. 1. Plasma free haemoglobin and LDH levels.

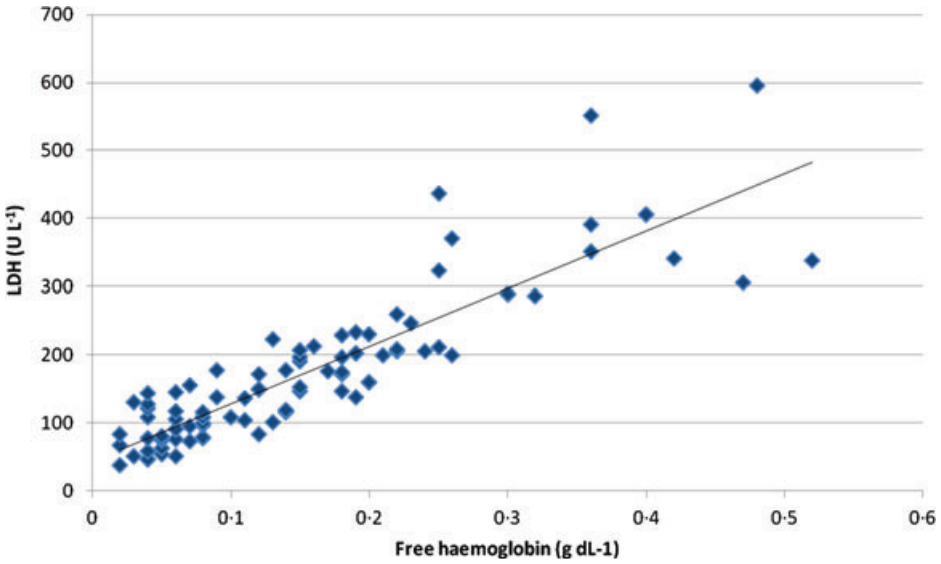

Fig. 2. LDH and plasma free haemoglobin results where plasma free haemoglobin is less than 1 g dL^{-1}.

but has a deviation of 0·2 g dL^{-1} at such low values. This would not provide valid and reproducible detection when the average free Hb is 0·3 g dL^{-1}. In an ideal situation QC samples would be analysed using sensitive test methods or specifically designed analysers, such as the HemoCue® Plasma/Low Hb System (HemoCue, Angelholm, Sweden) which can detect down to 0·0 g dL^{-1}. However, difficulties with sourcing such analysers, along with purchase costs of the unit and the corresponding cuvettes/reagents, and processing time constraints, will unfortunately mean this option is not suitable for most users of ICS.

Due to these reasons, alternative tests need to be identified that are cheap and readily available to users. Two such tests that are being proposed in this article are the lactate dehydrogenase (LDH) test and the Haemolysis Index.

LDH is an enzyme widely distributed in almost all body cells. In health only a small amount is detected, but is released from the cells into the bloodstream when cells are damaged or destroyed. Because of this, the LDH test can be used as a general marker of injury to cells.

The Haemolysis Index is a simple colourimetric test involving pre-diluting the blood sample with saline and reading the red colour of haemolysis using colourimetry. It may be used by laboratories as a measure of quality indices assessing haemolysis levels in samples that may interfere with laboratory tests.

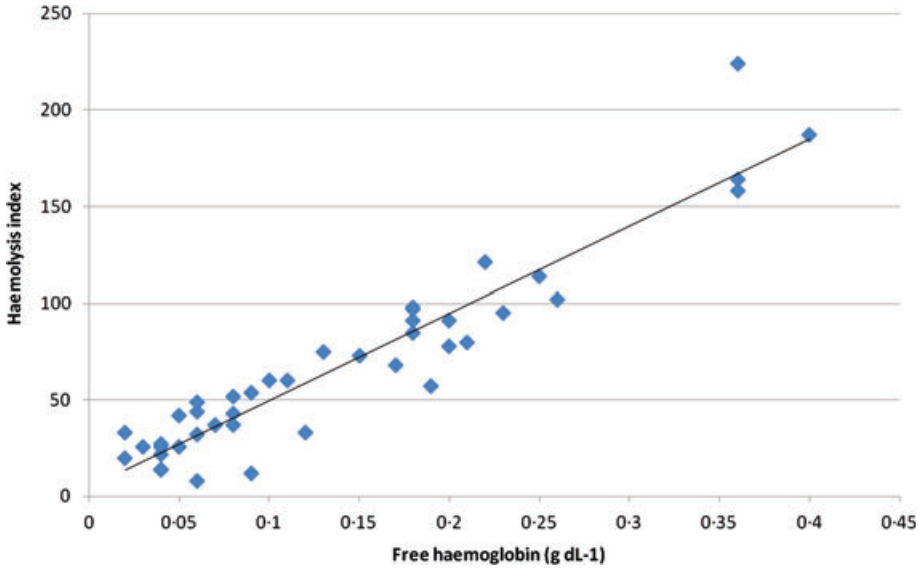

Fig. 3. Plasma free haemoglobin levels and Haemolysis Index.

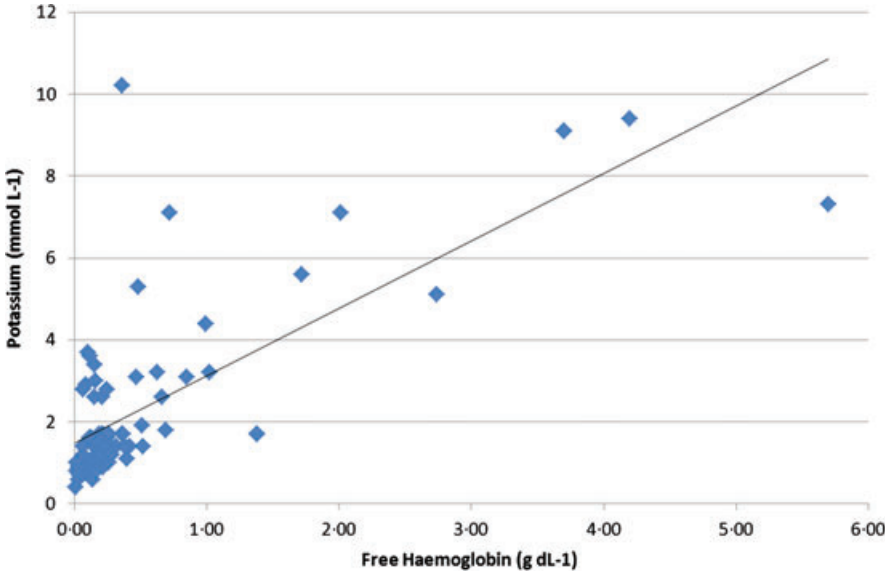

Fig. 4. Plasma free haemoglobin levels and potassium from ICS samples.

Aim

The aim of the project was to explore the potential use of LDH and the Haemolysis Index as markers of haemolysis, on comparison to the HemoCue Plasma/Low Hb System.

MATERIALS AND METHODS

Ethics Committee opinion was sought, but approval was not required.

As part of ongoing QC process within the Royal Cornwall Hospital, blood was collected from salvaged blood and allogeneic saline, adenine, glucose, mannitol (SAGM) leucodepleted red cell units. Samples were centrifuged at $1000 \times g$ and the supernatant processed on a HemoCue Plasma/Low Hb System (HemoCue, Angelholm, Sweden) and a Hitachi Modular P800 chemistry analyser (Roche Diagnostics, West Sussex, UK) for LDH and Haemolysis Index.

Data was collected from a total of 100 samples for plasma free haemoglobin and LDH analysis, however, due to unexpected analyser problems throughout this investigation data was only available from 44 cases when assessing Haemolysis Index.

Of the 100 samples, 55 were from allogeneic red cell units and 45 were from ICS. Mean age of allogeneic units 21 days (SD 10), range 4–42. All ICS samples were taken in the clinical setting from orthopaedic (primary and revision hip surgery),

vascular and obstetric (caesarean section) cases using either a Haemonetics Cell Saver® 5+ (Haemonetics Ltd, Watford, UK), Haemonetics OrthoPat® (Haemonetics Ltd) or Dideco Electa (Sorin Group UK Ltd, Gloucester, UK). ICS samples were taken post-wash after running through an IV giving set filter (170 microns) via a three-way tap. No needles were used in the collection of ICS samples, as this is a potential cause of haemolysis.

Statistical analysis

Correlation analysis was performed using Pearson's correlation or Spearman's correlation.

RESULTS

There was a very strong correlation between plasma free haemoglobin and LDH (0·960), and plasma free haemoglobin and the Haemolysis Index (0·944). Correlation between LDH and the Haemolysis Index was 0·996. Figure 1 demonstrates the correlation between all LDH and plasma free Hb levels; Fig. 2 focuses on plasma free Hb levels less than 1 g dL^{-1}, which are levels usually obtained from allogeneic and ICS samples (RCHT data). Figure 3 demonstrates the correlation between plasma free Haemoglobin and Haemolysis Index.

DISCUSSION

We have shown that the LDH and Haemolysis Index tests are suitable and reliable alternatives for measuring haemolysis from samples obtained from ICS or allogeneic blood. There was a very strong correlation between both tests and the plasma free haemoglobin result obtained from the HemoCue Plasma/Low Hb System.

The LDH test should be routinely available in all hospitals, with costs less than £0·50 and can be processed in a relatively quick time period. The Haemolysis Index test is even cheaper, but may have issues which will limit its ability to be routinely used. This test is analyser manufacturer dependent and may only be offered by large laboratories. There are a number of laboratories that do not measure quality indices such as the Haemolysis Index, and with no national requirement to do so. We suggest that if this test is to be included into a Hospital's QC package, discussions are held with the laboratory first to assess availability.

Previous suggestions have been that potassium may be a suitable test for ICS quality (Szpisjak *et al.* 2000; Personal communication J. Faulds). From data within our Institute, we identified that there was not a consistent strong correlation between potassium and free haemoglobin levels from combined allogeneic and ICS, with a correlation of −0·16. On breakdown into allogeneic and ICS, the correlation was slightly higher in blood obtained from ICS (correlation 0·69) compared to 0·4 in allogeneic blood, this being due to potassium leakage during storage rather than increased haemolysis. Figure 4 demonstrates the correlation of potassium with plasma free haemoglobin from ICS samples.

In conclusion, we have incorporated the LDH test into our Hospital's ICS QC package and recommend that this test be considered for all ICS QC samples. Due to potential availability issues with the Haemolysis Index we propose that this test is not routinely run as part of the UKCSAG QC package but may be performed by individual hospitals to collect further data and enhance the understanding of haemolysis in ICS blood.

ACKNOWLEDGMENTS

I. J. S. performed the research, analysed the data and wrote the paper. J. N. F. provided advice and reviewed the paper. Funding for Laboratory reagents and secondment to undertake this investigation was kindly provided by the Cornwall Haematology Charitable Funds and The Cornwall Leukaemia Research Fund, courtesy of Drs RS Noble and AR Kruger, Haematology and Blood Transfusion Department, Royal Cornwall Hospital Trust.

CONFLICT OF INTEREST

The authors have no competing interests.

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Szpisjak, D.F., Edgell, D.S. & Bissonnette, B. (2000) Potassium as a surrogate marker of debris in cell-salvaged blood. *Anesthesia and Analgesia*, **91,** 40–43.

Appendix 6

Sullivan, I.J., & Faulds, J.N. (2014). Assessment of intra-operative cell salvage haemolysis in the obstetric and orthopaedic clinical setting, in comparison with allogeneic blood. *Transfusion Medicine,* 24(5), 280-285.

Contribution by I Sullivan:

Design including literature search

All laboratory analysis

Manuscript writing

Number of citations:

Google Scholar:	6
Pubmed:	0
Web of Science:	1
Clarivate Analytics:	1

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Transfusion Medicine | ORIGINAL ARTICLE

Assessment of intra-operative cell salvage haemolysis in the obstetric and orthopaedic clinical setting, in comparison with allogeneic blood

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SUMMARY

Objectives/Background: Haemolysis is still a re-occurring theme in intra-operative cell salvage (ICS) with further haemolysis possibly caused by suction pressure, washing/centrifuging process and aspiration method. Previous investigations, along with manufacturer's reports, state that between 75 and 95% of free haemoglobin (Hb) is removed by the washing and centrifugation process; however, if these results are above the expected levels, excess free Hb may remain after washing.

The aim of this article was to quantify haemolysis levels whilst employing different aspiration methods from skimmed (orthopaedics) and pooled (obstetrics) surgery types and comparing this to allogeneic blood.

Methods/Materials: Samples obtained from 50 allogeneic units and 50 ICS cases (25 obstetric and 25 orthopaedic) were tested for plasma free Hb levels.

Results: Free Hb testing as a marker of haemolysis was greatest in orthopaedic 17·2 g L^{-1} (range: 1·7–57·0 g L^{-1}), obstetric 2·8 g L^{-1} (range: 1·0–13·5 g L^{-1}) and allogeneic 0·95 g L^{-1} (range: 0·2–4·8 g L^{-1}) cases.

Conclusion: ICS involving skimming collection techniques (orthopaedics) had significantly more haemolysis than pooled collections (obstetrics) ($P < 0{\cdot}001$). Further analysis of orthopaedic data highlighted a difference between the three machines used with the Haemonetics OrthoPat (Haemonetics Ltd., Watford, UK) significantly higher with a free Hb of 29·8 g L^{-1} compared with the other two machines 6·7 g L^{-1} ($P < 0{\cdot}001$). On comparison of ICS blood to allogeneic blood, free Hb levels obtained from ICS were significantly higher ($P < 0{\cdot}001$).

Key words: allogeneic, cell salvage, haemolysis, obstetric, orthopaedic.

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The advantages and benefits of intra-operative cell salvage (ICS) are well known with 2,3-diphosphoglycerate levels twice that of allogeneic blood and normal 24 h survival rates (Burman *et al.*, 2002; Hansen & Seyfried, 2011). However, haemolysis is still a re-occurring theme with further haemolysis possibly caused by the vacuum pressure or the washing/centrifuging process.

Similarly, the collection of salvaged blood appears to play a crucial part in the degree of haemolysis. The spilled orthopaedic surgical blood tends to be of a gradual loss, suctioning red cells from bone and tissue in a 'skimming' fashion. This causes mechanical trauma as they pass nonendothelial or mesothelial surfaces, as well as coming into contact with lytic enzymes, activated coagulation factors, fat, bone and surgical contaminants such as cement (Mortelmans *et al.*, 1994). In obstetrics or vascular surgery there is more of a pooling of spilled blood. When collected, these red cells are not exposed to the same trauma as seen in skimming.

The volume and rate of blood collected is an important factor when salvaging blood. It has been suggested that lower vacuum pressures produce less haemolysis, especially in post-operative drains (Munoz *et al.*, 2011), although higher vacuum rates are sometimes requested by surgeons to clear the surgical operating field rapidly (J. Faulds, 2014, personal communication). Within our cell salvage training programme we actively set the vacuum between 100 and 150 mmHg for all cell salvage machines, although this may be increased for short periods to support clearing the surgical site.

Previous investigations, along with manufacturer's reports, state that between 75 and 95% of free haemoglobin (Hb) is removed by the washing and centrifugation process (Levy *et al.*, 2001; Valbonesi *et al.*, 2001; Hansen *et al.*, 2008; Haemonetics Product Brochure, 2012a,b). However, if the results are above the levels expected, excess free Hb may remain after washing and therefore infusing large volumes of ICS blood may compound comorbidities due to the total load of free Hb (Sloan *et al.*, 2009).

Free Hb as a result of haemolysis released into the circulation is bound by haptoglobin which is then removed by the reticuloendothelial system, with haptoglobin being able to bind up to

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1·25 g Hb per litre of plasma (Hoffbrand *et al.*, 2001). Unbound free Hb is filtered by the glomerulus and reabsorbed in proximal tubule cells. When in excess, or insufficient haptoglobin to bind to the free Hb, free Hb becomes nephrotoxic resulting in circulatory free Hb in the plasma. When levels exceed reabsorption rates in the kidneys, it presents in urine as haemoglobinuria (Dacie & Lewis, 1992; Rother *et al.*, 2005). Acute renal failure could theoretically occur in severe episodes of haemoglobinuria caused by re-infusions of haemolysed ICS blood. This however does not seem to occur in the clinical setting possibly due to the low volumes returned in most surgical collections.

Free Hb is a scavenger of nitric oxide (NO) irreversibly binding to and reducing levels. NO is a regulator of smooth muscle tone and platelet activation and acts as a vasodilator in the microcirculation (Rother *et al.*, 2005; Ley *et al.*, 2012). Reduced NO levels result in vasoconstriction, systolic and diastolic blood pressure (BP) increases, reduced tissue oxygenation, smooth muscle dystomies, gastrointestinal (GI) contractions, endotheliac dysfunction and platelet aggregation with increased thrombotic events (Rother *et al.*, 2005; Sloan *et al.*, 2009; Kelleher *et al.*, 2011; Vermeulen Windsant *et al.*, 2012). Haemolysis also results in the release of enzymes that can affect NO synthesis (Rother *et al.*, 2005).

Previous investigations on the scavenging effect on NO by free Hb demonstrated a clear correlation ($P = 0{\cdot}002$) in patients who received allogeneic blood transfusions such that as circulating free Hb levels increased, NO consumption also increased. As expected, patients transfused with more allogeneic units had an increased free Hb level post transfusion, increasing to a maximum after 120 min before decreasing. Patients who had lower pre-transfusion haptoglobin levels also had higher free Hb levels (Vermeulen Windsant *et al.*, 2012).

As well as NO scavenging, the heme part of Hb breakdown can increase procoagulant effects resulting in increased platelet activation and increased release of inflammatory substances (Rother *et al.*, 2005; Sloan *et al.*, 2009). Damaged cells also cause coagulation abnormalities and other inflammatory morbidities including acute lung injury (Kelleher *et al.*, 2011).

It has been previously suggested that ICS can only reduce free Hb levels to $0{\cdot}3\,g\,L^{-1}$ as further haemolysis will occur during the processing and that levels may increase during machine use (Hansen *et al.*, 2004).

The aim of this investigation was to assess the quantity of haemolysis due to different aspiration collection methods in ICS: orthopaedics and obstetrics, and to add this data to the already published knowledge. Data was compared with that collected from allogeneic blood.

MATERIALS AND METHODS

Data was collected from 50 ICS cases and from 50 allogeneic units. Ethics Committee opinion was sought, but approval was not required. Data from this article has been presented at the 2013 Network for Advancement of Transfusion Alternatives (NATA) symposium and the 2013 British Blood Transfusion Society (BBTS) annual conference (Sullivan & Faulds, 2013a, 2013b).

ICS samples

Only the final product that would be re-infused to the patient was analysed. Pre-wash samples were not analysed. All ICS samples were taken in the clinical theatre setting, with all ICS machines operated in the automatic mode. Partial bowls were excluded from this investigation. Partial bowls from ICS is a debated subject with concern on the washout efficiency of contaminants and the quality of the final product, although the evidence for this concern is limited. The role of partial bowls is beyond the scope of this article and will be addressed in a separate report.

Post-wash samples were collected into ethylenediaminetetraacetic acid (EDTA) after running through an IV giving set filter (170 μm) via a three-way tap. As has been previously identified as the potential cause of haemolysis, and is therefore recommended, no needles or excessive pressure were used in the collection of samples or transferring of samples into the respective tubes. Sample tube tops were removed prior to gently running the blood down the side of the tubes.

Patients

Samples were obtained from 25 patients undergoing elective caesarean section. In 21 cases, blood was collected into a Haemonetics Cell Saver5+ incorporating SmartSuction-HarmonyAutoregulating Surgical Suction (Haemonetics Ltd., Watford, UK), whereas in the remaining four cases blood was collected into a Dideco Electa (Sorin Group UK Ltd., Gloucester, UK) using an integrated vacuum device.

From the 25 orthopaedic cases, the majority were primary total hip replacement (THR) ($n = 18$) or revision hip surgery ($n = 5$). A Haemonetics OrthoPat(Haemonetics Ltd.) using piped wall vacuum was used in 10 primary THR and 1 revision hip surgery, the Dideco Electa was used in 3 primary THR and 2 revision hip cases, with the Cell Saver 5+ used in 5 primary THR and 2 revision hip surgeries. The remaining two cases were one revision total shoulder replacement and ORIF fracture distal femur, both using the OrthoPat.

Allogeneic samples

All units tested were leucodepleted and stored in Saline, Adenine, Glucose, Mannitol (SAGM). Samples were either taken from wasted or time expired units that were still within the cold chain criteria, or a blood sample was taken from the unit giving set prior to connecting to a patient's cannula. No needles were used. Blood was collected into EDTA. Average age of the blood was 21 days.

In order to compare haemolysis levels from allogeneic and ICS levels to what could be expected in the general population, data were collected from 30 samples within our Institute and tested for plasma-free Hb levels.

Table 1. Median haemoglobin, haematocrit and free haemoglobin levels; (IQR)

	Sample size	Median Hb (IQR), min – max ($g\,L^{-1}$)	Median HCT (IQR), min – max ($L\,L^{-1}$)	Median free haemoglobin (IQR), min – max ($g\,L^{-1}$)
ICS all	50	165 (59·5), min – max: 113 – 262	0·502 (0·164), min – max: 0·314 – 0·744	4·65 (15·4), min – max: 1·0 – 57·0
ICS orthopaedic	25	197 (102), min – max: 113 – 262	0·557 (0·263), min – max: 0·314 – 0·744	17·2 (28·6), min – max: 1·7 – 57·0
ICS obstetrics	25	156 (31), min – max: 130 – 209	0·460 (0·107), min – max: 0·371 – 0·662	2·8 (2·6), min – max: 1·0 – 13·5
Allogeneic blood	50	197 (23·0), min – max: 163 – 219	0·656 (0·061), min – max: 0·513 – 0·728	0·95 (1·4), min – max: 0·2 – 4·8

min – max, minimum – maximum.

Sample analysis

All samples were processed immediately to avoid any storage changes. Samples were analysed on an Advia2120 Haematology System (Siemens UK, Surrey, UK) to obtain product Hb and haematocrit (Hct). To quantify free Hb levels, samples were centrifuged at $1000 \times g$ for 5 min and supernatant tested on a HemoCuePlasma/Low Hb System (HemoCue Ltd., Derbyshire, UK).

To assess if any further haemolysis occurred in the laboratory due to centrifuging samples for obtaining the supernatant, centrifugation experiments were performed. Samples were obtained from six ICS cases on separate occasions (two primary THR and four from elective caesarean section) and were distributed evenly into separate tubes and centrifuged at speeds ranging from 110 to $1700 \times g$, duration times between 2 and 5 min using braked and unbraked systems. Supernatant was then analysed for plasma-free Hb levels, using the HemoCue method stated above.

Statistical analysis

Parametric data was analysed using the *t*-test sample; non-parametric data was analysed using the Median test or the Mann – Whitney test. $P < 0{\cdot}05$ is considered statistically significant.

RESULTS

The initial centrifugation experiment showed no difference in haemolysis (plasma-free Hb levels) when subjected to varying centrifugal speeds and duration times; however, low centrifugal speeds of $110 \times g$ were insufficient to separate the supernatant from the red cells needed for testing.

Table 1 lists the median Hb, haematocrit and free Hb levels along with the inter-quartile range and minimum – maximum values from ICS and allogeneic units.

Free Hb levels were noted to be greatest in orthopaedics. On comparison of skimmed (orthopaedics) to pooled (obstetrics) free Hb levels were significantly higher in orthopaedic samples ($P < 0{\cdot}001$). Median pooled free Hb level was $2{\cdot}8\,g\,L^{-1}$ with a median skimmed level of $17{\cdot}2\,g\,L^{-1}$.

On further analysis of the orthopaedic data there was a difference between the three machines used, with the OrthoPat significantly higher with a free Hb of $29{\cdot}8\,g\,L^{-1}$ compared with the other two machines $6{\cdot}7\,g\,L^{-1}$ ($P < 0{\cdot}001$).

On comparison of ICS blood to allogeneic blood, free Hb levels obtained from ICS were significantly greater than those obtained from allogeneic blood ($P < 0{\cdot}001$), with the range ($0{\cdot}2 – 4{\cdot}8\,g\,L^{-1}$) similar to other published ranges (Szpisjak *et al.*, 2000; Burman *et al.*, 2002; Vermeulen Windsant *et al.*, 2012).

As the concentration of free Hb in the final ICS post-wash product will be affected by the total volume re-infused, it is often more appropriate to know the final amount of free Hb that would be re-infused to the patient. This is known as the dose and is useful when comparing products with different haematocrits. It can be calculated by multiplying the supernatant volume by the free Hb concentration (Haemonectics Corporation, 2008). Table 2 shows the total free Hb dose from ICS and allogeneic units from this study.

Comparison of skimmed to pooled ICS collections, the total free Hb dose was significantly higher ($P < 0{\cdot}001$). On breakdown of the orthopaedic data, and again consistent with the free Hb concentration reported, the total Hb dose was greater from the OrthoPat 1·26 g when compared with the other two machines of 0·72 g.

Free Hb levels as a representation of what could be expected in the general population within our Institute, had a median free Hb of median $0{\cdot}3\,g\,L^{-1}$ [interquartile range (IQR): 0·2; minimum – maximum 0·1 – 0·6].

DISCUSSION

Free Hb levels obtained from our orthopaedic cases are greater than other published data [range: $0{\cdot}75 – 3{\cdot}73\,g\,L^{-1}$ (Roberts *et al.*,

Table 2. Total free Hb dose from ICS and allogeneic units from this study

	Median free Hb dose (IQR), min – max (g)
ICS orthopaedic	0·94 (0·91), min – max: 0·09 – 6·20
ICS obstetrics	0·29 (0·39), min – max: 0·10 – 1·37
Allogeneic	0·10 (0·13), min – max: 0·02 – 0·44

min – max, minimum – maximum.

1991; Mortelmans *et al.*, 1994; Spain *et al.*, 1997; Cuby *et al.*, 2001)] but a direct correlation cannot be made because of limited case numbers and differences in the type of surgery and laboratory methodology.

On assessment of the higher levels obtained from the use of the OrthoPat in our investigation, no definitive conclusions can be made as the case numbers were limited, although this finding is consistent with a recent report with free Hb levels from the OrthoPat ranging from 4·0 to 67 g L^{-1} (Baxter *et al.*, 2012).

Data can only be found from one obstetric report with a free Hb level of 1·6 g L^{-1} (Fong *et al.*, 1999). There are however several reported cases from ICS use in cardiac surgery with levels ranging from 0·1 to 3·1 g L^{-1} (Kling *et al.*, 1988; Spain *et al.*, 1997; Cuby *et al.*, 2001; Kelleher *et al.*, 2001; Levy *et al.*, 2001; Burman *et al.*, 2002; Serrick *et al.*, 2003). Blood lost during cardiac surgery is more likely to pool in a manner similar to obstetrics.

Free Hb levels that could be expected in the general population with median 0·3 g L^{-1} are marginally higher than other reports with levels up to 0·1 g L^{-1} (Crosby & Dameshek, 1951; Hanks *et al.*, 1960; Mortelmans *et al.*, 1994; Sloan *et al.*, 2009), but this could be due to collection, storage or transport issues, or the testing methodology used. However, these normal ranges are significantly less than the reported free Hb levels in allogeneic and ICS blood ($P < 0{\cdot}001$).

Reviewing the published data on total free Hb doses in the re-infused product, the only reported ICS case in obstetrics identified a free Hb dose of 0·103 g (Fong *et al.*, 1999). In the study by Spain *et al.* (1997), as the average re-infused volume was highest in vascular surgery, it had the highest total free Hb dose of 1·15 g per patient. In their cardiac cases, the average dose was approximately 0·60 g per patient, and in the orthopaedic 'skimming' setting, with the lowest returned volume, the total dose per patient was approximately 0·3 g.

In a recent study exploring the fragility of cell salvaged blood, washed and unwashed post total knee arthroplasty, it was demonstrated that there was more haemolysis and damage in the washed group compared with the unwashed group as shown by a higher free Hb and higher mechanical fragility index. Washed blood, similar to methods we have used in our institution had a total free Hb dose of 0·55 g but this was not statistically different ($P = 0{\cdot}615$) when compared with unwashed ones (Ley *et al.*, 2012).

On comparison of the data obtained from our Institute to the available published data, free Hb concentrations and doses from pooled collections are consistent; however, our orthopaedic data is greater than those reported. This we feel warrants further investigations and is part of an ongoing quality assurance work within our Institution.

Although we have statistically demonstrated a difference, it does not address the clinical significance of this and the other results and at what level of re-infusing free Hb would this become significant. This could be far more difficult to assess, however due to variances between individuals. For example, patients with active inflammation may have increased haptoglobin levels and therefore have the potential to clear the free Hb faster. In addition, certain clinical situations will complicate the assessment of re-infusing free Hb/haemolysed blood so any changes in the BP or renal function, for example, will not be easily distinguished between the surgical procedure and ICS blood re-infused. It has been suggested that 0·1 g L^{-1} free Hb significantly impairs NO metabolism, levels greater than 0·16 g L^{-1} are linked to renal injury/dysfunction and greater than 1·0 g L^{-1} may cause haemoglobinuria (Widman, 1989; Reiter *et al.*, 2002). Theoretically a patient's haptoglobin levels would be sufficient to cover approximately 3 g of free Hb transfused before haemoglobinuria occurs, equivalent to 10 donor units with haemolysis levels of 0·5% (Sowemimo-Coker, 2002).

Within orthopaedics, an alternative system is the reinfusion of unwashed blood from post-operative drains. The quality of this blood is debated because it is known to have higher haemolysis levels (Munoz *et al.*, 2004, 2011), yet there have been many cases where blood from post-operative drains has been infused with higher levels of haemolysis without any harm being evident (Blevins *et al.*, 1993; Ayers *et al.*, 1995, Munoz *et al.*, 2011). This may be due to sufficient haptoglobin being present to absorb a significant amount of free Hb. It has been suggested that there is enough haptoglobin in the circulation to cover up to 1500 mL reinfusion of unwashed blood, whilst washing reduces the haemolysis further (Munoz *et al.*, 2004, 2011). Levels of haptoglobin have not been shown to decline faster in those receiving such unwashed blood compared with no cell salvage (Sebastian *et al.*, 2000). As such the importance of small amounts of infused free Hb is uncertain.

In order to ascertain the complete clinical significance of free Hb infusions, the dilutional effect of re-infusing into a total blood volume and the circulatory haptoglobin levels, it would be beneficial to measure haptoglobin and free Hb levels in the patient pre and post re-infusion, as well as assessing the re-infused product. There are very few case reports where this has been performed, with Mortelmans and colleagues showing an increase post re-infusion from 0·08 to 0·58 g L^{-1}. The final ICS post-wash product had a concentration of 1·77 g L^{-1} (Mortelmans *et al.*, 1994).

On the basis of our findings and the available data, we feel we are unable to comment completely on the significance of reinfusing haemolysed blood as we have analysed only post-wash samples. There were however no complications noted in any of the ICS re-infusions, and on review of our 50 patients' post-operative renal function there were no new cases of renal dysfunction noted by assessment of electrolytes, urea and creatinine.

In conclusion, this report has confirmed that haemolysis of varying degrees (at least 1 g L^{-1}) is still present in the final product that could be re-infused to the patient. This would agree with previous reports in that further haemolysis will still occur during the processing and use of ICS machines. Free Hb levels

obtained from autologous and allogeneic units were higher than those found in the normal population, with increased levels in ICS when compared with allogeneic blood. The significance of this is still unclear. Owing to the elevated results obtained in this current study, we feel that free Hb levels need to be addressed further and we will be repeating this study, including analysis of free Hb and haptoglobin levels and renal function in patients pre- and post-reinfusion.

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I.J.S. performed the research, analysed the data and wrote the article. J.N.F. obtained samples, provided advice and reviewed the article.

CONFLICT OF INTEREST

The authors have no competing interests.

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Appendix 7

Teare, K.M., Sullivan, I.J., & Ralph, C.J. (2015). Is cell salvaged vaginal blood loss suitable for re-infusion? *International Journal of Obstetric Anesthesia*, 24(2), 103-110.

Contribution by I Sullivan:

- Design including literature search
- Data collection
- Data analysis
- Manuscript writing

Number of citations:

Google Scholar:	12
Pubmed:	1
Web of Science:	5
Clarivate Analytics:	1

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ORIGINAL ARTICLE

Is cell salvaged vaginal blood loss suitable for re-infusion?

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ABSTRACT
Background: Haemorrhage is one of the commonest causes of maternal critical care admission. Cell salvage used during caesarean section can contribute to a reduction in allogeneic blood consumption. This study sought to provide a practical method to salvage blood lost after vaginal delivery and a description of the constituents before and after washing.
Methods: Blood lost after vaginal delivery was collected from 50 women and washed in a cell salvage machine. No blood was re-infused to any patient in this study. The following measurements were made pre- and post-wash: haemoglobin (haematocrit), alpha-fetoprotein, albumin, lactate dehydrogenase, plasma free haemoglobin, heparin concentration, fetal red cells and identification of bacterial species and colony-forming units (cfu).
Results: Median haemoglobin concentration post-wash was 15.4 g/dL. Alpha-fetoprotein, lactate dehydrogenase and albumin concentrations were significantly reduced post-wash (<1 KU/L, 183 IU/L, 0.011 g/L, respectively; P <0.001). Median fetal red cell level post-wash was 0.15 mL [range 0–19 mL]. Median bacterial contamination concentration post-wash was 2 cfu/mL, with a median total count of 303 cfu.
Conclusions: Vaginal blood can be collected efficiently with little disruption to patient management. The amounts of haemolysis and washout of non-red cell blood components are consistent with results in our cell salvage quality assurance programme for caesarean section and non-obstetric surgery. Although bacteria are detectable in all the post-wash and post-filter samples, the median residual contamination is similar to that found with cell salvage in caesarean section, and if re-infused would result in a circulating bacteraemia of <1 cfu/mL; this is similar to that seen with dental procedures (0.3–4.0 cfu/mL) and is thought to be clinically insignificant.

Keywords: Bacteria; Blood transfusion; Autologous; Obstetrics; Intra-operative cell salvage; Postpartum haemorrhage

Introduction

The Confidential Enquiry into Maternal and Child Health reports have consistently identified haemorrhage as an important direct cause of maternal death.[1] It is one of the commonest reasons for maternal critical care admission, and obstetric patients are significant users of allogeneic blood products.[2] There are risks associated with donor blood transfusion which include acute transfusion reaction, lung injury and, although rare, the possibility of death from transfusion error, and transmission of infection which may have serious long-term consequences. These risks are monitored by annual Serious Hazards of Transfusion (SHOT) reports.[3] The morbidity from blood transfusion also includes postoperative infection; the risk increasing with each unit transfused.[4] Allogeneic blood is an increasingly scarce and expensive resource, and in the UK a well-developed blood transfusion service exists to minimise risk. In some countries there is difficulty supplying allogeneic blood, and in addition patients may refuse allogeneic transfusion on religious grounds. Blood conservation strategies, including cell salvage, aim to reduce consumption of allogeneic blood.

In current obstetric practice, the use of cell salvage is generally restricted to caesarean section. A 2012 survey of UK obstetric units found that 47% had cell salvage equipment, with frequency of use varying between units.[5] Cell salvage was introduced at The Royal Cornwall Hospital Trust (RCHT) in 2008 and is now used routinely at caesarean section. The number of obstetric patients who receive allogeneic blood per delivery in our unit has reduced from 1.8% in 2008 to 0.8% in 2013, with a reduction in the mean number of units transfused per patient from 3.3 to 1.9. In 2013, however, 81% of women who received allogeneic blood delivered vaginally, not by caesarean section. There is currently no evidence to support or reject the use of cell salvage after vaginal delivery. This study aimed to test the feasibility and effectiveness of a method to salvage vaginal

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blood loss, with a description of constituents before and after washing in a cell salvage machine.

Methods

This descriptive study assessed blood salvaged by a cell saver after vaginal delivery in a series of 50 participants. The study was approved by the National Research Ethics Service Committee Southwest: Plymouth and Cornwall [12/SW/0136]. All participants gave written informed consent. The study was conducted at the RCHT. No cell-salvaged blood was re-infused to any participant.

Inclusion criteria were vaginal delivery and an estimated blood loss of $\geqslant$200 mL after transfer to the operating theatre for clinical reasons, which included on-going blood loss, instrumental delivery, manual removal of placenta and perineal tear repairs. Women whose blood loss was managed entirely in delivery rooms were excluded.

Collection was carried-out by the clinical team using the equipment shown in Figs. 1 and 2. After delivery of the baby, the obstetrician placed an under-buttock drape with pouch (Steri-Drape™ 1084, 3M Health Care, Bracknell, UK) to collect blood. Similar drapes are commonly used in UK maternity units and non-obstetric theatres. The aspiration and anticoagulation line was lowered into the pouch. Approximately 200 mL of heparinised saline (30 000 U in 1000 mL) was run rapidly into the pouch and the rate then adjusted to a slow drip. Once surgical treatment was complete, the blood/heparinised saline mix was aspirated into the reservoir and processed with a Cell Saver® 5+ Autologous Blood Recovery System (Haemonetics Ltd., Coventry, UK). Processing was performed in the automatic mode with a wash volume of 1500 mL, double the manufacturer's recommended setting. This is the same setting used in our hospital at caesarean section, and had been decided before introduction of the obstetric cell salvage service. In all cases a single aspiration line was used.[6]

Samples of salvaged blood were taken from: (1) the collection reservoir labelled 'pre-wash'; (2) the re-infusion bag labelled 'post-wash' and (3) after passing the full volume through a leucodepletion filter (RS1 VAE Pall Medical, Portsmouth, UK) labelled 'post-filter'. At all stages the blood was agitated gently before sampling to ensure even mixing. The following measurements were made at each stage:

Fig. 1 Position of under buttock drape

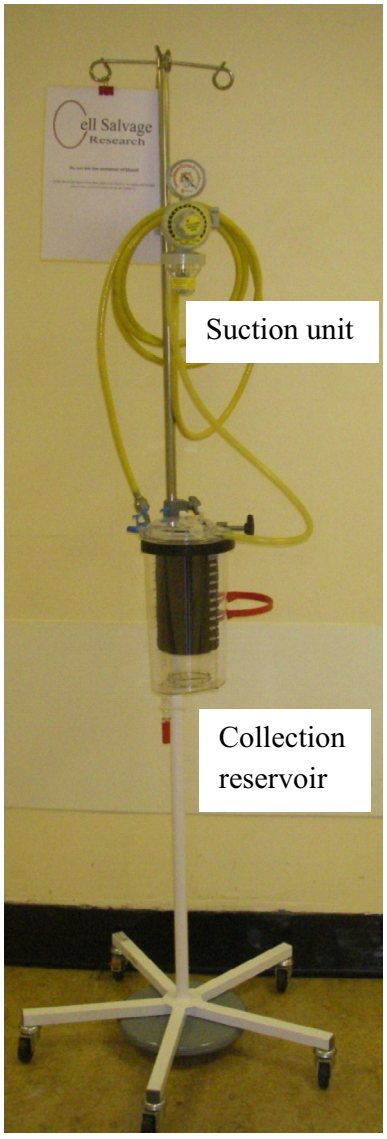

Fig. 2 Collection drip stand with reservoir and suction

1. Full blood count: haemoglobin concentration (Hb) and haematocrit (Hct) were analysed on an Advia® 2120 Haematology System (Siemens UK, Surrey, UK).
2. Alpha-feto protein concentration (AFP), used as a marker of amniotic fluid contamination, was processed on a Hitachi Modular P800 chemistry analyser (Roche Diagnostics, West Sussex, UK).
3. Fetal red blood cells (fetal RBCs) using the Kleihauer-Betke test.
4. Micro-albumin, as a marker of washing efficiency, was processed on a Hitachi Modular P800 chemistry analyser (Roche Diagnostics, West Sussex, UK).

5. Heparin level using the anti-Xa test was processed on an Instrumentation Laboratory TOP Coagulometer (Instrumentation Laboratory, Warrington, UK)
6. Plasma free haemoglobin concentration (free Hb), as a marker of haemolysis, was measured using a HemoCue® Plasma/Low Hb System (HemoCue Ltd, Derbyshire, UK). To aid assessment of haemolysis, lactate dehydrogenase (LDH) was also measured. It has been shown to be a reliable indicator of haemolysis in cell salvage, allowing comparison with haemolysis concentrations from other cell salvage techniques.[7]
7. Bacterial contamination: aerobic and anaerobic enriched blood cultures were inoculated with 5 mL each, collected into standard aerobic (BacT/ALERT SA) and anaerobic (BacT/ALERT SN) bottles and processed using the Blood culture BacT/ALERT Microbial Detection System (bioMérieux Ltd., Hampshire, UK). A separate 1 mL sample was spread on an agar plate and incubated for 12 h, after which a colony count was performed according to standard procedures.

The percentage of red cells available to re-infuse was calculated only for those 31 women who had a drape placed immediately after delivery (i.e. those who had an instrumental delivery in theatre, rather than gave birth in a delivery room and subsequently moved to theatre). The calculation was:

1. Maternal venous preoperative Hct multiplied by estimated blood loss. Clinicians used graduated markings on the collection reservoir and pictures of blood soaked swabs and receivers to estimate blood loss.
2. Post-wash salvaged blood Hct multiplied by its volume.

Washout efficacy of the cell salvage machine was calculated for LDH, AFP, albumin and heparin as pre-wash minus post-wash divided be pre-wash expressed as a percentage. The last maternal venous blood sample taken before delivery was tested for Hb/Hct along with fetal red cell levels using the Kleihauer-Betke test.

Bacterial contamination (species and quantity) of cell salvage blood was also measured in 20 patients undergoing emergency caesarean section. Post-wash salvaged blood was sampled from the re-infusion bag and tested using the same methods.

Statistical analysis

Non-parametric data are presented as median and range or interquartile range (IQR). Non-parametric data analysis was performed using Wilcoxon Signed Ranks Test.

Results

Fifty-seven women were recruited. The first seven collections were processed in a 70 mL bowl with poor and unexpected washout rates; those results were excluded from further analysis. The 125 mL bowl was used for the remainder of the study.

A series of 50 participants is presented (Table 1). The 50 participants were delivered by instrumental or normal vaginal delivery; 48 had singleton births and two delivered twins. Nine women received an allogeneic blood transfusion (median 1 unit; range 1–3 units). In 48 cases the wash volume was 1500 mL. In two cases the machine automatically increased the wash volume to 1815 mL and 2500 mL respectively, as the sensors detected inadequate washing. From the 50 cases, 34 were from complete bowls, the remaining 16 were from partial bowls, defined as a centrifuge bowl that was not completely filled with the cell salvage machine in automatic mode. Laboratory Data from all 50 cases are presented (Table 2). Table 3 shows median [IQR] values from the full bowls only.

Median pre-wash volume was 800 mL [IQR 700–1200 mL]. Median post-wash volume was 133 mL [IQR 118–204 mL].

In 31 women, the drape was placed immediately after delivery; in the others there was delay whilst transferring to theatre. For those 31 women a median of 28% [IQR 21-35%] of red cells lost were available to re-infuse. The washout efficiency of the cell salvage machine is shown by a median washout reduction rate of 95% for LDH and 99% for AFP, albumin and heparin (Table 2). Albumin was significantly reduced post-wash (P <0.01) with no further significant reduction post-filter (P=0.77). Albumin levels post-wash were similar to levels collected from caesarean section patients with a maximum of 0.13 g/L detected post-wash, equivalent to a 99.9% wash-out rate.

Alpha-fetoprotein, used as a marker of amniotic fluid, was significantly reduced post-wash (P <0.01), consistent with previous investigations.[6,8] The maximum level detected post-wash was 8 KU/L, and this was due to the pre-wash sample having a very high concentration of AFP initially; this still represented 99.9% wash-out efficiency. There was no further significant reduction

Table 1 Patient characteristics

Age (years)	30 [26–35]
Parity	1 [1–1]
Body mass index at booking (kg/m^2)	25.5 [22.5–29.5]
Gestation (weeks)	40 [40–41]
Estimated blood loss (mL)	700 [500–1500]
Pre-delivery haemoglobin (g/dL)	12.3 [11.3–13.0]
Post-delivery haemoglobin* (g/dL)	9.3 [8.4–10.3]

Data are median [IQR]. *Values following transfusion, when required.

Table 2 Constituents of salvaged blood

	Pre-wash	Post-wash	Post-filter
Haemoglobin (g/dL)	3 [1.9–4.4]	15.4 [15.3–17.9]	11.2 [6.0–15.3]
Haematocrit	0.087 [0.054–0.1]	0.461 [0.314–0.5]	0.333 [0.167–0.5]
AFP (KU/L)	62 [23–294]	<1 [1–3]	<1 [1–1]
LDH (IU/L)	374 [239–762]	183 [125–350]	87 [47–192]
Albumin* (g/L)	11 [6–13]	0.011 [0.005–0.030]	0.013 [0.005–0.021]
Heparin (IU/mL)	6.3 [5.9–6.5]	0 [0–0]	0 [0–0]
Free haemoglobin (g/dL)	0.13 [0.07–0.3]	0.1 [0.04–0.25]	0.11 [0.03–0.38]
Fetal RBCs (mL)	0.28 [0.02–1.4]	0.15 [0.04–1.99]	0.08 [0.002–0.77]

Data are median [IQR]. *Albumin derived from micro-albumin test; AFP: alpha-fetoprotein; LDH: lactate dehydrogenase; RBC: red blood cells.

Table 3 Constituents of salvaged blood from full bowls only

	Pre-wash	Post-wash	Post-filter
Haemoglobin (g/dL)	3.3	16.4	14.5
Haematocrit	0.102	0.490	0.439
AFP (KU/L)	78	<1	<1
LDH (IU/L)	526	259	142
Albumin* (g/L)	11	0.011	0.013
Heparin (IU/mL)	6.3	0	0
Free haemoglobin (g/dL)	0.14	0.13	0.13
Fetal RBCs (mL)	0.26	0.61	0.15

Data are median and derived from 34/50 full bowls. *Albumin derived from micro-albumin test; AFP: alpha fetoprotein; LDH: lactate dehydrogenase; RBC: red blood cells.

post-filter (P=0.82). Lactate dehydrogenase was significantly reduced post-wash (P <0.01) and post-filter (P <0.01). The reason for the reduction in LDH post-filter while other contaminants were not reduced is unclear. Plasma free Hb levels were reduced post-wash but the reduction was not statistically significant (P=0.40), and there was no further reduction post-filter (P=0.61). Heparin was significantly reduced post-wash (P <0.01). It was detected in 4/50 samples post-wash. Three of the four positive results were from partial bowls, and contained a median of 0.14 IU/mL which equated to a total dose of 16 IU. If re-infused this would be clinically insignificant. There was no further significant reduction of heparin post-filter (P=0.90).

Fetal red cells were detected in all except three cases. The median volume of fetal red cells post-wash was 0.15 mL [range 0–19 mL] (Fig. 3). Maternal peripheral blood samples, taken between two hours and two days before delivery, showed fetal red cells were already present in 13 cases (26%): median level 0 mL; [IQR 0–0.48 mL], with a maximum fetal red blood cell volume of 3.4 mL.

Post-wash bacteria counts were significantly reduced (P <0.001) (Table 4). Bacteria were detected in all the post-wash and post-filter samples (median residual contamination 2 cfu/mL). The predominant bacteria identified in this study were *Escherichia coli*, *Enterococcus* sp. and coagulase-negative staphylococci which were likely to be part of the genital or skin flora (Table 5). At emergency caesarean section, post-wash cell salvaged blood had detectable bacteria in all 20 samples (Table 4).

Discussion

The study has shown that blood lost vaginally can be collected in an efficient manner with minimal impact or disruption to the clinical team or patient management. The drapes did not dislodge, and there were no episodes of blood running outside the pouch. The collection system was simple and quick to set up. A dedicated member of staff is not required to operate the collection system, although a trained member of staff must be part of the team.

Blood collections were confined to theatre in order to limit training requirements to a small team. Collection in delivery rooms is possible after training midwives, although most women with ongoing bleeding are transferred to the operating theatre for further management. Midwives could collect blood using a drape included routinely in all delivery packs: in our hospital this would cost an additional £1.50 per pack. Heparinised saline can be poured into the drape by a midwife as soon as excessive bleeding is recognised. Aspiration of blood to the cell salvage machine reservoir does not need to be immediate and can be performed when a trained person arrives. In our institution this would be an operating department practitioner or theatre nurse. The costs of disposable items for the cell salvage machine (approximately £90) are only incurred when excessive and on-going bleeding occurs, and these costs are recovered if transfusion of only one unit of blood is avoided.

Results from this study are consistent with those obtained from caesarean section and vascular cases using intra-operative cell salvage at the RCHT.[6] Unpublished data from caesarean section with full bowls post-wash have median values of Hb 15.3 g/dL; Hct 0.455; LDH 236 IU/L; albumin 0.0064 g/L; and fetal RBC 0.6 mL. The 16 cases from partial bowls had a lower and variable post-wash Hb [IQR 5.9–10.3] and Hct [IQR 0.170–0.312]. Median post-wash volume of the partial bowls was 118 mL [IQR 115–122 mL]. This is

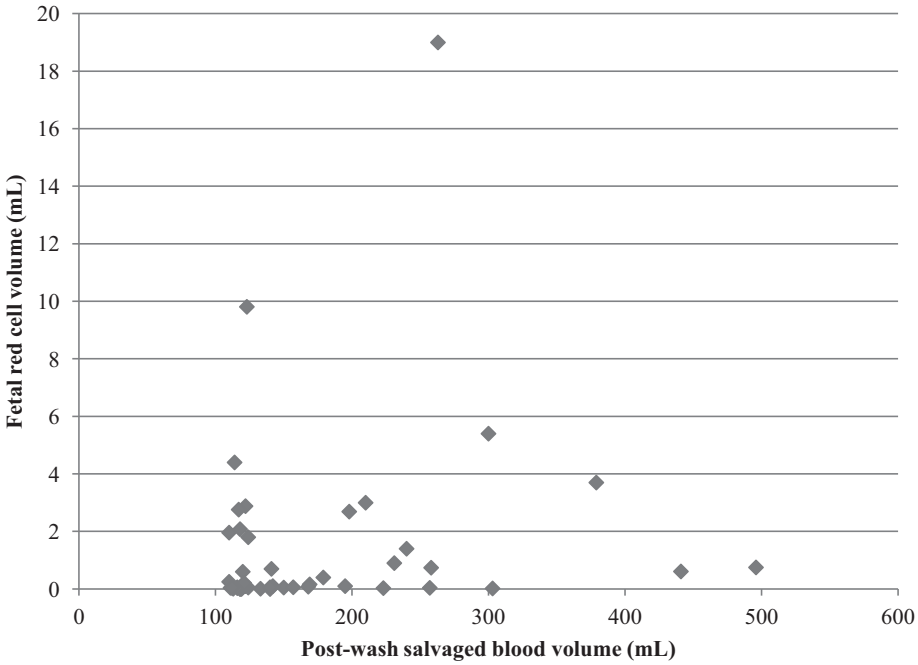

Fig. 3 Fetal red cell volumes detected in post-wash samples

Table 4 Bacterial counts from vaginal delivery and caesarean section

	Vaginal delivery*			Caesarean section
	Pre-wash	Post-wash	Post-filter	Post-wash‡
Bacterial concentration (cfu/mL)	8 [1–84]	2 [1–25]	3 [1–14]	1 [1–1]§
Total bacterial count (cfu)	3400 [1278–52200]	303† [188–1245]	438† [98–2115]	

Data are median [range]. *Data from 32 patients. †P <0.001 compared to pre-wash.; §Maximum value 30 cfu/mL.; ‡Median post-wash volume 165 mL.

Table 5 Species of bacteria detected from partial and full bowls.

	Vaginal delivery		Caesarean section
	Pre-wash (n=50)	Post-wash (n=50)	Post-wash (n=20)
*Escherichia coli**	34	39	1
Enterococcus spp.*	33	36	3
Enterobacter cloacae	0	0	1
Coagulase-negative staphylococcus	14	11	16
Staphylococcus saprophyticus	2	0	0
Staphylcoccus aureus	0	0	1
Lactobacillus spp.	5	3	0
Mixed anaerobes	4	3	0
Coliforms	2	1	0
Alpha haemolytic streptococci	2	1	2
Group B streptococci*	2	1	0
Haemolytic streptococci mixed (Non-A, B, C)	1	2	0
Diptheroids	2	0	1
Proteus*	1	1	1

Data are numbers. *Organisms found in the three vaginal samples with the highest bacterial counts.

to be expected as in the absence of sufficient red cells the machine tops-up the bowl with saline. The role of partial bowls from cell salvage in caesarean section is currently unclear.

As this is the first study to attempt collection of vaginal blood loss, only women who bled 200 mL or more were studied. This volume of blood loss was chosen as the amount likely to be sufficient to fill a 125 mL centrifuge bowl. However, 200 mL may not be sufficient for processing in all cases because it is dependent on the maternal Hct, degree of haemolysis and the amount of blood lost on swabs.

Red cell haemolysis occurs during suction and centrifugation in the cell salvage process and produces free Hb which was not removed by washing. Samples taken post-wash revealed less haemolysis than that seen at the same stage in caesarean section and far less than that in orthopaedic surgery. Levels were similar to that found in allogeneic blood.

Fetal red cell contamination was expected. Both the quantity and range of data, including higher outlying values (19 mL in this study), are consistent with previous reports involving caesarean sections.[6,9–11] The reason for the outlying value is not known. The presence of fetal red cells in the maternal circulation is an important consideration for red cell antigen incompatibilities between mother and fetus. Rhesus incompatibilities have been significantly reduced by routine prophylactic anti-D treatment throughout pregnancy,[12] but other antibodies to red cell antigens can result in fetal hyperbilirubinaemia and anaemia in future pregnancies. Clinically relevant antibodies that have been implicated in haemolytic disease of the newborn include anti-K, anti-c, anti-Fy(a), and anti-Jk(a).[13] However, there is also a risk of alloimmunisation of the mother either from allogeneic blood or a sensitising event during pregnancy. A total of 26% of women in this study had fetal red cells present in venous samples at some time before delivery. Fetal red cells are known to be present in the maternal circulation before delivery, with approximately 50–78% of samples being positive in the third trimester.[14,15] During delivery further contamination of the maternal circulation is likely. Contreas et al. found 50% of maternal blood samples had detectable fetal red cells after delivery.[16] We do not know if transplacental exposure to fetal red cells occurred in all participants because postnatal maternal samples were not tested. In the absence of transplacental exposure, re-infusion of salvaged blood could present a risk of alloimmunisation.

Our previously published study from 26 participants undergoing caesarean section reported the fetal cell count as a percentage.[6] Reviewing those data and calculating the fetal red cell contamination in mL, the median value was 0.57 mL [IQR 0.34–0.82] with a minimum of 0.05 mL and maximum of 5.57 mL. There was poor correlation between fetal cell contamination and post-wash blood volume (correlation=0.368).[6] These are similar to findings in the current study. We continue to investigate alloimmunisation rates following re-infusions of salvaged blood during caesarean section and fetal red cell contamination levels present in the maternal circulation.

Although bacteria are detectable in all the post-wash and post-filter samples, the median post-wash residual contamination (2 cfu/mL) was similar to that in cell salvage in caesarean delivery (1–1.3 cfu/mL).[10] The median dose that would be re-infused would be 303 cfu (cfu mL x post-wash volume) which, when diluted by the maternal circulation, would result in a circulating bacteraemia of <1 cfu/mL. This is similar to that seen in asymptomatic bacteraemia during dental procedures (0.3–4.8 cfu/mL)[17] and is likely to be clinically insignificant.

Waters et al. assessed bacterial contamination in caesarean section patients and detected a median post-wash concentration of 1.3 cfu/mL [IQR 0–4 cfu/mL]. Samples from the maternal iliac vein were found to contain the same species of bacteria.[10] Of the organisms identified by Waters 93% were staphylococci. Median concentrations of bacteria identified in the current investigations (post-wash) were similar, although the range of concentration was larger. The current study found that blood salvaged at caesarean section also contained bacteria. The median quantity was similar to that in vaginal cases, although the upper range was higher in vaginal cases. Species were the same as found in vaginally salvaged blood but with a predominance of skin organisms.

Women who receive salvaged blood after emergency caesarean delivery have similar rates of postoperative infection to those who do not.[18] Cell salvage washing can reduce bacterial load in contaminated blood. In one in-vitro study, expired allogeneic blood was inoculated with known quantities of bacteria and colony counts performed after washing. A mean reduction of 99% was found for *E. coli*.[19] Studies of blood salvaged from apparently sterile surgical sites have found bacterial contamination rates of 12.7–33.3%. In penetrating abdominal trauma, 91.7% were contaminated.[20] Studies of between 18 and 152 patients have examined the relationship between re-infusion of contaminated blood and postoperative infection. Recipients included liver transplant and penetrating abdominal trauma patients.[20] No association with postoperative infection has been found.[20] All studies involved surgery where broad spectrum antibiotics are given routinely.

In this study the leucodepletion filter added no benefit, and bacterial concentration appeared to increase with the use of a filter. This may be an artefact of passing small volumes through a filter that retains some red cells. Median Hb post-wash (pre-filter) was 15.4 g/dL, which reduced to 11.2 g/dL post filter. Our series includes low volume blood loss that many clinicians would be unlikely to consider re-infusing. The volume of washed blood was further reduced by removing samples for testing before using the filter. The effect of the filter retaining some

red cells would effectively increase the concentration of all other components including bacteria. This may not be an issue with higher volume bleeds where the fixed volume of the filter becomes less significant.

Most women in the study did not experience a post-partum haemorrhage, but were included to make the study possible. As our unit has 4700 deliveries per year, to include only major vaginal haemorrhages ($\geqslant$2000 mL) would require prolonged recruitment. Having completed this study we now know that it is possible to collect vaginal blood loss without impeding surgical management, but this was not known at the outset. We failed to demonstrate a relationship between fetal red cell contamination and volume of salvaged blood. We believe smaller bleeds can be valid proxies for severe haemorrhage as we would not expect total quantities of amniotic fluid contamination, fetal red blood cells and bacteria to increase as the volume of maternal blood loss increases.

This is the first study to assess the practicality of salvaging vaginal blood, and efforts were not made to maximise the red cell volume collected. There was no training period for staff to practise collection, and cases where collection had been sub-optimal were included. There were two cases in which the heparinised saline was not switched on until some minutes into collection, and swabs were not washed. Appropriate training and washing swabs are likely to increase red cell recovery. Blood lost in delivery rooms was not included; 19 women who delivered out of theatre lost blood before starting the collection in theatre. The delay between collection and washing of salvaged blood was due to a researcher being called to complete processing of the collection. This delay was an issue unique to the study and does not occur in clinical use at caesarean section. In practice the washing and processing of the collection would start, and usually be completed, during the operative procedure.

Quantitative bacterial analysis was obtained from only 32 out of 50 cases and limited in sensitivity from 1–100 cfu/mL. When 10 mL blood samples were tested in enriched cultures, bacteria were present in all cases; samples reported as $<$1 cfu/mL were analysed as 1 cfu/mL. Three post-wash samples were reported as $>$100 cfu/mL, and have been rounded to 100 cfu/mL for analysis although the true upper limit of bacterial contamination was not established.

In conclusion, blood lost vaginally can be salvaged. Following washing in the cell saver, blood composition is similar to that re-infused after caesarean section for Hb, haemolysis, efficiency of washout of amniotic fluid and heparin. Fetal red cells are present in volumes no greater than in blood from caesarean section. Bacteria are present in blood to be re-infused, although the quantity is low and unlikely to increase the risk of infection. The actual risk of post-reinfusion infection has not been established. Re-infusing salvaged vaginal blood loss is a treatment option the authors would consider in life-threatening haemorrhage in women who cannot have allogeneic transfusion. A trial of cell salvaged blood re-infusion versus allogeneic blood transfusion after vaginal birth is needed.

Disclosure

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Appendix 8

Sullivan, I.J., & Ralph, C.J. (2018). Obstetric intra-operative cell salvage and maternal fetal red cell contamination. *Transfusion Medicine,* 28(4), 298-303.

Contribution by I Sullivan:

Design including literature search

Data collection

Data analysis

Manuscript writing

Number of citations: This has just been recently published

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Transfusion Medicine | ORIGINAL ARTICLE

Obstetric intra-operative cell salvage and maternal fetal red cell contamination

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SUMMARY

Background: The significance of fetal red blood cell (RBC) contamination in obstetric intra-operative cell salvage is not fully known. It is unclear if we re-infuse a larger volume of fetal RBCs into the maternal circulation than the amount that occurs secondary to transplacental haemorrhages is unclear. We also do not know if there is a critical volume required to cause alloimmunisation or if larger volumes increase the risk.

Objectives: The aim of this study is to provide data on the level of fetal RBC contamination in the maternal circulation prior to delivery and immediately post-partum and to compare these levels to those found in processed cell-salvaged blood.

Methods: In the first part of this study, we quantified the levels of fetal RBCs circulating in women immediately prior to delivery. This was then repeated with a separate group measuring the levels of fetal RBCs pre- and post-delivery.

Results: We found that 37% of women had fetal cells detected in their circulation, median 0·00 mL (IQR 0 – 0·24; average 0·3 mL, maximum 4·56 mL). Fetal RBCs were present pre-delivery (maximum 0·66 mL) in 16% of women, increasing to 53% post-delivery (median 0·66 mL; IQR 0·22 – 2·20, maximum 21·20 mL).

Conclusions: We have shown that fetal RBCs are present in the maternal circulation throughout pregnancy and that the volumes are comparable to that obtained from intra-operative salvage, with contamination amounts of up to 19 mL. At the Royal Cornwall Hospital, our experience and evidence supports offering intra-operative salvage to all women, and we have not noted an increase in antibody formation, compared to allogeneic transfusion.

Key words: alloimmunisation, cell salvage, fetal RBCs, obstetric.

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Fetal red blood cells (RBCs) are known to be present in the maternal circulation throughout the ante-natal and peri-natal periods. However, the available published data to define the range of circulating fetal RBCs are limited to small case numbers and are outdated (Klein & Anstee, 2014).

Our previous projects on the use of cell salvage in obstetrics, both in caesarean sections and vaginal deliveries, showed that fetal RBCs are collected during the cell salvage process (Sullivan *et al.,* 2008; Teare *et al.,* 2015). Due to the similarities between fetal and adult RBCs, in size and density, the cell salvage machines cannot differentiate between them, and therefore, any fetal RBCs aspirated will be washed, filtered and returned to the woman together with her own RBCs.

The significance of this contamination is not known and has not been systematically investigated. It is unclear, e.g. what follows if we re-infuse a larger volume of fetal cells into the maternal circulation than the amount that occurs secondary to transplacental haemorrhages in normal pregnancy. It is also unclear, at least in this setting, if there is a critical volume of fetal RBCs required to cause alloimmunisation or if progressively larger volumes of fetal RBCs increase the risk of alloimmunisation. Furthermore, there is no evidence to show how these rates of alloimmunisation compare to those following an allogeneic blood transfusion.

The formation of anti-D in a woman with an RhD-positive baby, although clinically significant, has been reduced by the use of routine prophylactic anti-D treatment throughout pregnancy. However, fetal hyperbilirubinaemia and anaemia in future pregnancies can still occur when antibodies have been formed to other mismatched red cell antigens, e.g. following the formation of anti-K, anti-c, anti-Fy(a) and anti-Jk(a) (Murphy *et al.,* 2013).

When using obstetric cell salvage (ICS) during a caesarean section, there is a need to be aware of the risk of formation of antibodies other than anti-D. This fact is not widely appreciated, and many investigators involved in obstetric ICS are still only concerned with the risk of anti-D. If ICS is to be routinely employed in obstetrics, the formation of other clinically significant antibodies that could impact future pregnancies must be considered, and potential risk must be addressed by multidisciplinary teams.

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At the Royal Cornwall Hospital (RCH), we have now re-infused ICS blood to more than 900 women undergoing a caesarean section with no significant clinical complications. We have shown that it is a cost-effective and feasible procedure and has reduced the rate of allogeneic transfusion in what is a young and healthy population (Ralph *et al.,* 2009; Ralph *et al.,* 2011). We have also shown that it is feasible to collect blood for re-infusion from vaginal deliveries (Teare *et al.,* 2015).

The pathophysiology of an amniotic fluid embolus (AFE) and its association with obstetric ICS is uncertain but remains a theoretical risk. The clinical diagnosis of AFE is usually one of elimination and is now considered to be a type of anaphylactic reaction rather than an embolic disease. Furthermore, the washing and filter processes used in cell salvage have now been shown to effectively remove amniotic fluid contaminants, fetal squames and other debris (Catling *et al.,* 1999; Waters *et al.,* 2000; Sullivan *et al.,* 2008). We feel that fetal RBC contamination is perhaps the only barrier to what could be a valuable routine service offered to all parturients.

The aim of this study was to measure the level of fetal RBC contamination in the maternal circulation prior to delivery and during the delivery process and to compare these levels to those found in processed, cell-salvaged blood.

MATERIALS AND METHODS

We quantified the levels of fetal cells in 100 women immediately prior to delivery using a visual microscopic counting method based on differences of solubility properties in acid conditions of fetal haemoglobin (HbF) and adult haemoglobin (Kleihauer-Betke Technique). To improve the accuracy and precision of these measurements, we repeated this experiment in a further 100 women pre- and post-delivery, measuring the RhD antigen by indirect immunofluorescence and flow cytometry.

Estimation of fetal RBC in maternal circulation pre-delivery

A total of 100 women attending the Delivery Suite at RCH were randomly selected for fetal RBC estimation. As the fetal blood group was unknown, counts had to be performed using the Kleihauer–Betke method, which was performed following British Society for Haematology (BCSH) guidelines (Austin *et al.,* 2009). Testing was performed on the pre-delivery blood sample taken as part of the routine admission to the Delivery Suite. As no additional samples were required and all data were anonymised, Ethics Committee opinion was sought, but specific approval was not required. No women receiving ICS were included.

Estimation of fetal RBC in maternal circulation pre- and post-delivery

A further 100 RhD-negative women who gave birth to an RhD-positive baby were tested using a BRAD-3-FITC monoclonal anti-D (*Source*: IBGRL, NHSBT Filton, UK) by flow cytometry, counting 500 000 cells on a FACSCanto flow cytometer (BD Biosciences, Oxford, UK). Women were not excluded based on their ABO status. No women receiving ICS were included. *Post-partum* samples were taken approximately 30-45 min post-delivery, as per BCSH guidelines (Austin *et al.,* 2009).

Data were also collected on previous pregnancies, gestation, estimated blood loss and the maternal and fetal blood groups. The strength of an antibody screen pre-delivery due to prophylactic anti-D [negative – no prophylactic anti-D detected pre-delivery, weak (<2+ grading) or strong (>2+ grading)] was also assessed for any correlation between fetal RBC levels and antibody strength. The grading criteria were based on the results obtained from the pre-delivery group sample and screen sample when tested on an Immucor NEO Blood Grouping Analyser (Norcross, GA, USA) in combination with the grading scores used by UK NEQAS Blood Transfusion (West Hertfordshire Hospitals NHS Trust, UK).

Statistical analysis

Parametric data are presented as mean and standard deviation (sd). Non-parametric data are presented as median (IQR and/or range). Data were analysed using the Mann–Whitney test, Kruskal–Wallis test and Spearman's rank test as appropriate.

RESULTS

Estimation of fetal RBC in maternal circulation pre-delivery

From the 100 samples analysed pre-delivery, the average gestation was 39 weeks (sd 1·8), with the majority of women between at 37 and 40 weeks gestation (IQR 38–40). Six women delivered between 32 and 36 weeks, and nine women exceeded term.

The average estimate of fetal RBC in the maternal circulation was 0·3 mL (IQR 0·0–0·24; median 0·0 mL). Of the 100 cases, 37 (37%) had fetal cells detected, with a maximum of 4·56 mL detected. The remaining 63 cases had no fetal cells detected when counting 25 low-power fields (as per guidelines); however, when examining the slide outside this area, eight were positive, with an occasional cell seen. This would be equivalent to a fetal contamination of less than 0·24 mL. From the 37 positive cases, 32 were less than 2 mL. The remaining cases were 2·16, 2·64, 2·86, 3·36 and 4·56 mL.

Estimation of fetal RBC in maternal circulation pre- and post-delivery

A further 100 samples were analysed. These samples were taken from RhD-negative women who gave birth to an RhD-positive baby. The median gestation of pregnancy was 40 weeks (range 34–42 weeks). Seven samples were taken from women who had exceeded term. The median parity was one (range 1–6), and median estimated blood loss was 400 mL (range 100–2300 mL).

Table 1. Reported fetal red blood cell contamination levels in obstetric ICS blood

Investigation	Number of cases	Findings
Blood salvage during caesarean section (Rainaldi *et al.,* 1998)	15	Three of 15 cases positive, 1·8 – 2% fetal RBC contamination level. Estimated maximum of 3 mL.
Cell salvage in obstetrics: an evaluation of the ability of cell salvage combined with leucocyte depletion filtration to remove amniotic fluid from operative blood loss at caesarean section (Catling *et al.,* 1999)	27	Average 9·6% fetal RBCRBC contamination level (range 2 – 19%)[1]
Clearance of fetal products and subsequent immunoreactivity of blood salvaged at caesarean delivery (Fong *et al.,* 1999)	10	All positive, mean 0·4% fetal RBC contamination level[2]
Amniotic fluid removal during cell salvage in the caesarean section patient (Waters *et al.,* 2000)	14	1·7% fetal RBC contamination level[2]
Contamination of salvaged maternal blood by amniotic fluid and fetal red cells during elective Caesarean section (Sullivan *et al.,* 2008)	26	Median 0·57 mL (range 0·05 – 5·57 mL)
The development and implementation of an obstetric cell salvage service (McDonnell *et al.,* 2010)	3	280 – 1600 cells in 50 low-powered fields
Is cell-salvaged vaginal blood loss suitable for re-infusion? (Teare *et al.,* 2015)	50	Median 0·15 mL (range 0 – 19 mL)
Royal Cornwall Hospitals Trust Quality Control Quality Control programme (our unpublished observations)	56	Median 0·39 mL (range 0 – 12·8 mL)

[1]The authors in this study reported fetal red cell contamination as a percentage; however, they commented that, based on average ICS volume of 100 mL, this would equate to 9·6 mL (range 2 – 19 mL). However, it appears that the haematocrit of the product may not have been taken into consideration, and the fetal contamination would be lower than this.

[2]These two investigations also reported fetal red cell levels as a percentage and therefore cannot be translated into straightforward volume contamination as the haematocrit and ICS blood volume need to be taken into consideration.

From the women for whom samples were taken pre- and post-delivery, there was no correlation between post-delivery fetal red cell contamination levels, number of previous pregnancies, gestational age or estimated blood loss (correlation −0·218, 0·076 and 0·170, respectively).

From these 100 samples, 72 women had vaginal deliveries and 28 delivered by caesarean section. We were unable to match the ABO groups due to the routine use of ICS for over 95% of our caesarean section patients. Many women received ICS re-infusions following a caesarean section, and this would have resulted in an increased rate of false positives, with positive cases post-delivery due to ICS and not the delivery process. This is discussed further below.

Of the 100 cases, 16 (16%) were positive pre-delivery (maximum 0·66 mL), with 53 cases (53%) positive post-delivery. Of the 53 positive samples tested post-delivery, the median fetal red cell level was 0·66 mL (IQR 0·22 – 2·20; maximum 21·20 mL).

Of the 72 women who had a vaginal delivery, 12 samples (17%) were positive pre-delivery and 38 (53%) were positive post-delivery. The median fetal cell level post-delivery in these 50 positive cases was 0·44 mL (IQR 0·22 – 1·42; range 0·22 – 12·96 mL). Of the 28 women who had a caesarean section delivery, four samples (14%) were positive pre-delivery, and 15 (54%) were positive post-delivery. Median fetal cell level post-delivery of the 15 positive cases that had a caesarean section was 1·32 mL (IQR 0·33 – 6·37; range 0·22 – 21·20 mL). There was no statistical difference between post-delivery fetal RBC contamination levels and whether delivery was by vaginal or caesarean section ($P = 0·377$).

From the 100 cases tested pre- and post-delivery, 81% were ABO compatible (i.e. no maternal ABO antibodies directed against fetal red cells). Post-delivery results were statistically higher in ABO-compatible deliveries ($P < 0·001$) (median 0·22 mL, mean 1·52 mL, range 0 – 21·20 mL in ABO compatible; median 0·00 mL, mean 0·92 mL, range 0 – 1·54 mL in ABO incompatible). This would suggest that fetal red cells are cleared faster from the maternal circulation in ABO-incompatible deliveries, confirming previous work summarised by Klein & Anstee, 2014.

Following the finding regarding ABO compatibility and fetal RBC levels, there was interest in whether the strength of prophylactic anti-D detected in the pre-delivery sample had any impact on fetal RBC levels post-delivery in maternal circulation. As such, the strength of the antibody screen and post-delivery contamination levels were examined. Unlike ABO incompatibilities, there was no statistical link between antibody strength of prophylactic anti-D detected in the pre-delivery sample and the level of fetal RBC contamination in the post-delivery sample ($P = 0·566$).

DISCUSSION

The current study has confirmed previous reports that fetal RBCs are present in the maternal circulation, with up to 37% of

woman having fetal cells present in their circulation pre-delivery, increasing to 53% post-delivery.

As for other published levels, Gordon & Bhoyroo, 1966, reviewed 42 cases and identified fetal RBCs present in the maternal circulation during the antenatal period from 8 weeks gestation through to labour, with levels increasing as the pregnancy progressed, with the highest volume in the third trimester; 18·8% of the cases were positive in the first trimester, increasing to 78% in the third trimester. This was attributed to the reducing efficiency of the ageing placenta towards the end of pregnancy (Gordon & Bhoyroo, 1966; Urbaniak, 1998). In addition, during the third trimester, the fetoplacental blood volume is the highest: 150 mL at 31 weeks gestation compared to 25 mL at 19 weeks gestation (Urbaniak, 1998). It has been concluded that the biggest risk of alloimmunisation would therefore occur after 28 weeks gestation (Urbaniak, 1998). The current study (testing from 34 weeks' gestation) and those of Mehta *et al.,* 1979 and de Wit *et al.,* 2011, (testing from 26 weeks) showed no correlation between fetal RBC volume and gestational age (correlation 0·165).

The report by Clayton, 1966 detected fetal cells in the maternal circulation in 40–50% of cases, with a later report by Bowman *et al.,* 1986, who studied 33 healthy patients and detected fetal RBCs of up to 0·06 mL prior to delivery, in the first and second trimester, increasing to a maximum of 5·15 mL in the third trimester. A total of 63·6% of their cases were positive post-delivery, range 0·05–26·6 mL. Only three cases post-delivery had fetal levels of greater than 1 mL. They concluded that up to 75% of the patients were exposed to fetal RBCs at some time during pregnancy, at labour or after delivery and also confirmed that the volume of bleed increased as pregnancy progressed.

A further investigation by Chhabra *et al.,* 2011, examined bleeding in the third trimester; 40% were positive pre-delivery, and of these cases, half had levels of less than 0·25 mL, 27·5%, 0·25–1 mL and 22·5% greater than 1 mL. Post-delivery, 56% were positive. However, these results cannot be compared directly to ours as they only studied bleeding cases due to ante-partum haemorrhages, placental abruption or unclassified haemorrhage.

From our recent investigation into the feasibility of using ICS from vaginal blood loss, all 50 patients included had a Kleihauer performed pre-delivery with 13 cases (26%) being positive (range 0–3·4 mL) (Teare *et al.,* 2015); this is consistent with the data collected from the current study.

Transplacental haemorrhage (TPH) is known to occur ante-natally outside known sensitising events, e.g. trauma. Peak TPH has been identified during delivery, again as a result of placental separation, resulting in the highest chance for fetal RBCs entering the maternal circulation. This has been confirmed by several reports, with one report commenting that 0·94% of patients at 30–39 weeks gestation have TPHs greater than 2·5 mL, and 50% have detectable fetal RBCs post-delivery. Of this 50%, 1% had a TPH greater than 3 mL and 0·3% 10 mL or more (Contreras, 1998). Another report stated that during the labour process 1% of women will have a TPH greater than 4 mL and 0·3% greater than 15 mL (Austin *et al.,* 2009).

From our results and the other studies listed above, there is evidence that 16–78% of women are exposed to fetal RBCs ante-natally, with levels of up to 5·15 mL, and more than half of all mothers are exposed to fetal RBCs post–delivery, with levels of up to 26·6 mL. But how does this compare to what is found in ICS?

Table 1 lists the fetal RBC contamination levels in obstetric ICS from published reports. The data need to be treated with caution as the number of reports and the sample size populations are low and the standards of testing and measurement vary. In some case reports, ICS was only used in large traumatic blood losses, and this is perhaps not a representative population of routine deliveries, therefore making it hard to justify any final comments. It does, however, demonstrate that fetal RBCs are present in the final re-infused ICS product in volumes of up to 19 mL, with no investigations finding values of fetal RBC higher than 19 mL in the maternal circulation pre- or post-delivery.

On reviewing the initial data from our previous study involving 26 cases undergoing caesarean sections (Sullivan *et al.,* 2008), we originally reported fetal red cell contamination in samples taken from the ICS reinfusion pack as a percentage. Revisiting this data and calculating the fetal red cell contamination volume, the median was 0·57 mL (IQR 0·34–0·82), with a minimum of 0·05 mL and maximum of 5·57 mL. There was no significant difference between the use of one suction and two suction devices ($P = 0·5334$). There was no correlation between fetal cell contamination and returned ICS volume (correlation = 0·368), and from our report on using ICS in vaginal blood loss, median fetal red cell contamination was 0·15 mL (range 0–19 mL) (Teare *et al.,* 2015*)*.

Current ongoing QC data (unpublished observations) within our institution of ICS blood obtained in caesarean section cases indicate that we have a median fetal red cell contamination of 0·39 mL (IQR 0·20–1·15; max 12·8 mL) of fetal RBC in the ICS re-infusion volume.

It is not known what volume is needed to provoke an antibody response. Will 1 mL do the same as 19 mL? Gunson *et al.,* 1970, calculated that 0·5 mL would be enough to initiate an anti-D response in healthy male volunteers, with another report stating that even 0·1 mL RBC may initiate an anti-D response (Jakobowicz *et al.,* 1972) The volume needed to provoke an immune response to other red cell antigens (e.g. K, Fy, Rh) is not known and would perhaps have to be greater than that needed for RhD due to the fact that RhD is the most immunogenic red cell antigen. The true volume required for sensitisation is unlikely to be quantified due to the complications involved in replicating a full human immune system *in vitro*. From the data we have collected at RCH, we have calculated the current risk of producing an antibody due to allogeneic blood transfusion is 0·35%. To rule out an increased rate of producing an antibody from receiving ICS blood with 95% certainty – that the upper 95% confidence interval is less than 0·45% – would require a total study size of 15 236 patients; the total sample is split

randomly, 7618 patients to receive allogeneic blood compared to 7618 receiving ICS blood, making this study not feasible.

Since 2012, RCH has established a 24/7 routine use of ICS in the obstetric operating theatre, using it in >95% cases and re-infusing most women who have a processed volume of blood. All those women who receive a re-infusion are followed up 3–4 months post-re-infusion to have a sample taken for antibody screening. To date, we have had two cases develop an antibody post-ICS (with no allogeneic transfusion). Both cases had a complicated obstetric history, one with repeated ante-natal bleeds and admissions from the beginning of the third trimester and the second with a twin vaginal delivery requiring urgent surgical intervention due to complications and bleeding (first baby was delivered vaginally with the second by caesarean section an hour later). As such, no definitive answers can be drawn, although it would suggest that ICS-processed blood does not put women at any more of a risk of antibody formation than women who receive an allogeneic transfusion. We strongly recommend that other institutions using ICS in obstetrics adopt this method of following up women 3–4 months post-re-infusion in order to collect data on alloimmunisation rates.

We also recommend, when possible, that to assist with data collection and assessment of fetal red cell contamination, samples should be taken post-delivery pre-ICS re-infusion for fetal red cell calculation (Kleihauer). However, it must be stressed that if a decision is made to undertake this testing, laboratories must be involved in initial discussions, and samples must be clearly marked as research only and not to be confused with post-natal samples from RhD-negative mothers for anti-D dosage (which must be taken post-re-infusion, as per guidelines). So far 6 of 16 samples (38%) that have been taken immediately post-delivery but before re-infusion of ICS blood have shown to be positive for fetal cells (range 0·24–6·2 mL).

In conclusion, we have shown that fetal cells are present in the maternal circulation ante-natally in 37% women and post-natally in 53% of women. Using this evidence, we offer ICS to all woman undergoing caesarean sections and re-infuse processed blood if available regardless of post-operative Hb estimation. We have not noted an increase in antibody formation in those women who have received a re-infusion of ICS blood but suggest continuing surveillance of antibody formation in these cases to determine the true rate of alloimmunisation after the use of ICS.

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I. J. S. performed the research, analysed the data and wrote the paper. C. J. R. assisted with writing the paper and provided advice.

CONFLICT OF INTEREST

The authors have no competing interests.

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Appendix 9

Sullivan, I.J., & Ralph, C.J. (2019). Obstetric intra-operative cell salvage: a review of an established cell salvage service with greater than 1000 re-infused cases. *Anaesthesia,* accepted for publication.

Contribution by I Sullivan:

Design including literature search

Data collection

Data analysis

Manuscript writing

Number of citations: This has just been accepted for publication.

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ORIGINAL ARTICLE

Obstetric intra-operative cell salvage: a review of an established cell salvage service with greater than 1000 re-infused cases *

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Summary

The use of cell salvage in obstetrics has been increasing steadily. There are now some concerns relating to cost and there remains a perceived risk of amniotic fluid embolus and of fetal red cell sensitisation. We present observational data of intra-operative cell salvage in obstetrics, alloimmunisation rates, quality of cell salvaged blood and consider the use of partial first bowls. Some of this data has been previously presented at meetings and published in abstract form.

From 2008 to the end of 2017, 1170 women have had a re-infusion of cell salvaged blood with no clinical safety concerns. Median returned volume 231ml (interquartile range 154-306ml), minimum 80ml, maximum 1690ml. Cell salvage was set up for collection for all category of caesarean sections in greater than 95% of cases.

Of the 1170 re-infused, 647 (55%) women have provided a follow-up sample for alloimmunisation testing, with two positive cases. We have not seen an increase in the dose of prophylactic anti-D being administered post-delivery.

This large data set demonstrates using cell salvage routinely as part of our obstetric patient blood management strategy has resulted in an overall reduction in the number of women transfused allogeneic blood and the amount of blood transfused. The quality of blood processed from partial first bowls is no worse than that from full bowls. We have not used the leucodepletion filter since 2015 and have not noted any adverse clinical events to date, either with or without the leucodepletion filter.

INTRODUCTION

The most recent Confidential Enquiries into Maternal Deaths (MBRRACE-UK) report covering the period 2013-15 has shown that obstetric haemorrhage is still a leading cause of maternal death, with no improvement in numbers over the last 20 years [1]. The Royal College of Obstetrics and Gynaecologists 'Green-top' guidance estimates that each year in the UK there are greater than 4000 cases of severe obstetric haemorrhage, with the majority receiving an allogeneic transfusion [2]. The most recent NHS Maternity Statistics report [3] for the period 2016-17 in England showed 27.8% of deliveries were by caesarean section and that rates increased with age and accounted for 44.1% of deliveries to women aged 40 and over. Of these women, 23.5% were having elective caesarean sections and 20.1% were emergencies. Women aged 40 and over also have the highest BMI at booking. All these are risk factors for bleeding and can result in increased allogeneic transfusion rates.

For operations such as caesarean section that are predicted to have a blood loss of more than 500 ml, the use of cell salvage is encouraged as an alternative to allogeneic blood [4]. However the recently updated Association of Anaesthetists of Great Britain and Ireland guidelines on cell salvage (July 2018) [4], no longer advocate routine use of cell salvage in obstetrics following the results of the largest multicentre randomised controlled study (SALVO) [5]. The availability of cell salvage in obstetric practice had been steadily increasing and a survey conducted in 2017 showed cell salvage was available in 84% of obstetric units, although only 50% of units had 24-h access and used it routinely [6]. There is still a perceived risk of amniotic fluid embolus despite there being no reported cases, and an unquantified risk of fetal red cell sensitisation resulting from the potential contamination of the aspiration and salvaged processed blood with fetal red blood cells. There are also concerns with issues relating to resources, staffing, training and resulting costs.

Multiple studies have found autologous blood (cell salvaged blood) to be a safer alternative to allogeneic blood; with adverse effects from cell salvaged blood quoted as 0.027% compared to 0.14% with allogeneic blood [7]. There are also increased risks of infections, morbidity and five-year mortality with allogeneic blood [8-14]. Recipients may be exposed to unknown pathogens in donated blood of which we are currently unaware, but in the future may be significant. Complications of allogeneic blood transfusion can increase length of stay rates by 1.3% per unit transfused [15]. Women who delivered at our hospital who have had an allogeneic transfusion are more likely to have a higher estimated blood loss and infections treated post-operatively, when compared to those who have had a re-infusion of cell salvaged blood, or no transfusions [15]. Women receiving cell salvaged blood are at lower risk of receiving an allogeneic blood transfusion and developing post-partum anaemia [16-17]. Our priority is to reduce exposure of women to allogeneic blood and consider alternatives when appropriate.

We present our practice and large observational database to demonstrate the routine use of intraoperative cell salvage and re-infusion of cell salvaged blood has provided a safe alternative to allogeneic blood, to over 1000 women. It is an important component of our obstetric blood conservation strategy which shows re-infusing cell salvaged blood has resulted in not only a reduced

risk of exposure to allogeneic blood, but also a reduction in the number of units of allogeneic blood transfused. We include data on follow-up alloimmunisation rates and quality of cell salvaged blood.

METHODS

We have performed a retrospective cohort study using data collected on our hospital databases. All of these are approved by the Caldicott Guardian. Ethics Committee and Research & Development approval was sought where required.

We use cell salvage routinely in our maternity operating theatre for all women having caesarean section, as well as women having major surgery such as post-partum laparotomy. We use a Cell Saver® 5+ System (Haemonetics Corporation, Braintree, MA, USA) which is dedicated to this location. The machine is set up and operated by an operating department practitioner (ODP), whose other duties are to assisting the anaesthetist. All ODPs are trained in cell salvage following a comprehensive competency-based training programme, including theoretical and practical based skills assessment.

The machine is initially set up for collection only. Since 2008 we have used a single specific wide-bore sucker without a Yankauer tip to collect blood into the reservoir [18], which is anticoagulated with a pre-prepared solution of saline containing 30,000 IU heparin.l^{-1}. The suction pressure is normally set at 150 mmHg but is increased to a maximum of 300mmHg in the presence of heavy bleeding. We routinely wash the swabs in saline to increase red cell return [19].

If the operating team judge that the patient has lost enough blood to require replacement and there is sufficient blood collected in the reservoir, then the blood processing system is opened, set up and the collection is processed with a double wash of 1500 ml saline for a 125 ml bowl. If, after processing in the automatic setting, the bowl is not filled to capacity with red cells, this is called a partial first bowl. Due to concerns with the washing efficiency of incomplete bowls, these partial first bowls of less than 125 ml red blood cells are not usually re-infused but discarded. Subsequent partial bowls can be topped up to 125mls, from processed red blood cells and are not discarded. With experience, the ODP becomes better able to assess which collections, when processed, will fill a 125 ml bowl.

The final decision to re-infuse the cell salvaged blood is made jointly by the obstetrician and anaesthetist, in discussion with the woman whenever possible. Re-infusions are offered to all women, regardless of the postoperative haemoglobin concentration. These are usually given in the operating theatre or immediately postoperatively in recovery. The blood is never removed from the patient's bedside, and it is discarded if not re-infused within six hours of the start of the collection. Routine observations are continued throughout the re-infusion as for allogeneic blood and per Trust protocol.

Blood warmers and pressurised bags are not used for re-infusion, which is administered by gravity alone. Re-infusions were all administered through a leucodepletion filter (Pall LeukoGuard® RS Filter; Pall Europe, Portsmouth, UK) until 2012. The use of filters was gradually reduced until 2015 when

they were removed, and blood was given through a standard blood giving set. Throughout the data collection period, this has been the only change to our cell salvage practice.

Data are collected for all cases where cell salvage is used, including pre-operative haemoglobin concentration, estimated blood loss, start time of collection, volume collected, volume of re-infusion, expiry time and patient and staff details. If processed cell salvaged blood is not re-infused, the reasons for this are documented. These are recorded on a monitoring sheet intra-operatively and then transferred to an electronic database. Any technical problems with cell salvage are noted on the Trust cell salvage monitoring forms and reported to the Blood Conservation Team, and manufacturer if applicable. Any adverse events relating to cell salvage are reported in accordance with the Trust Incident Reporting Policy, and where appropriate to relevant external bodies (Serious Hazards of Transfusion (SHOT) haemovigilance scheme; Medicine and Healthcare Products Regulatory Agency (MHRA)).

Transfusion practice throughout the data collection period has been restrictive, with a transfusion trigger of haemoglobin concentration < 70 $g.l^{-1}$, and review after each unit transfused.

Our Trust guidelines on management of major obstetric haemorrhage, in use throughout the period of data collection, have not changed with regards to the use of tranexamic acid.

Other blood conservation strategies such as ante-natal and post-natal treatment of anaemia have been implemented since the start of the data collection period. In 2012 the treatment of iron deficiency anaemia was changed from Venofer (Vifor International; Glattbrug, Switzerland) to a single total dose intravenous preparation (Ferrinject, Vifor International; Glattbrug, Switzerland). The number of women receiving ante-natal total dose intravenous iron has always been less than five women per year. Post-natally women with a haemoglobin value ≤ 80 $g.l^{-1}$ are offered total dose intravenous iron, or ≤ 95 $g.l^{-1}$ if symptomatic. Women with haemoglobin values above the threshold for total dose intravenous iron will be discharged home on oral iron.

To assess the risk of alloimmunisation due to fetal red cell contamination, consenting patients are invited to send a repeat blood sample three to four months after surgery to screen for any maternal antibodies.

Throughout the reported time period we performed quality control sampling on an ad hoc basis. Samples for quality control were taken from the re-infusion pack (post-wash, pre-giving set filter) to check the quality of blood being returned (haemoglobin and haematocrit), the amount of haemolysis (lactate dehydrogenase and plasma free haemoglobin tests), protein levels (microalbumin and potassium tests), heparin contamination and fetal red cell levels. Samples are collected after running through an IV giving set filter (170microns) via a three-way tap and not obtained using a syringe and needle which can cause haemolysis [20]. Sample tube tops are removed prior to gently running the blood down the side of the sample tube.

RESULTS

From April 2008 to the end of 2017, 1170 women have had a re-infusion of cell salvaged blood (figure one). All cell salvaged blood was collected in the operating theatre in all cases. Cell salvage was set up for collection for all caesarean sections (emergency and elective, category one to four) and post-partum laparotomies, being used in more than 95% of cases from 2012. Although each year the percentage of processed collections which are reinfused increases (from 53% in 2012 to 70% in 2017), the number of actual caesarean section cases reinfused blood is just over 30% of the cases (table one).

All re-infusions have been administered without clinically significant adverse events, with no cases of maternal collapse or hypotension seen either with or without the leucodepletion filter. The median (IQR [range]) volume of cell salvaged blood that was returned was 231 (154-306 [80-1690]) ml.

The rate of allogeneic transfusions to the whole obstetric population has fallen to 0.6% (0.4% of intra-partum women), and for women who have had a caesarean section it is 0.2%. In 2017, 71% of Obstetric allogeneic blood transfusions were given to women following a vaginal delivery, with 89% in 2016 and 95% in 2015. Table two lists the transfusion data from the last ten years.

Of the 1170 re-infused cases, 44 women also received an allogeneic red blood cell transfusion using 84 red cells in total. Twelve cases received FFP (49 units in total), two cases received a unit of platelet each and three cases received cryoprecipitate, each case having two units. One of these cases was a Jehovah's Witness who received two units of cryoprecipitate only.

Of the 1170 women re-infused, 509 (44%) have provided a sample, and on further checking of women who did not provide a sample, a further 138 had a group and screen taken at a later date ("incidental follow ups") for other reasons such as subsequent pregnancy, other hospital attendances, surgical/medical procedures, taking the total to 647 (55%). Two cases have returned a positive antibody screen.

Table three describes the cell salvage blood quality data. Data is also included from 'partial' first bowls from inadequate collection volumes, and allogeneic red blood cell units for comparison. As expected the haemoglobin and haematocrit were lower in partial bowls, but the plasma free haemoglobin, lactate dehydrogenase and potassium levels from full bowls were found to be higher ($p=0.000$, $p=0.000$, $p=0.000$ respectively), with albumin levels similar with no significant difference between full and partial bowls (p 0.861). Plasma free haemoglobin and lactate dehydrogenase levels from partial bowls were found to be similar to that of allogeneic blood, however levels from full bowls were higher than allogeneic, although free haemoglobin levels were not statistically significant ($p=0.826$, $p=0.000$ respectively). We have previously identified that there is more haemolysis in cell salvaged blood compared to allogeneic blood [20]. The pH of cell salvage blood is higher than that of allogeneic blood (mean from full bowls 7.55 (SD 0.09); partial bowls 7.39 (0.22); allogeneic units 6.85 (0.17).

Fetal red blood cells were present in nearly all cell salvaged samples tested. Median level from full bowls (n=104) was 0.59ml (0.17-1.48 [0.00-19.00]). Median levels from partial bowls (n=32) was 0.21ml (0.04-0.54 [0.00-3.83]). The difference between partial and full bowls was statistically significant (p=0.001).

DISCUSSION

We have presented data which demonstrates the routine use of cell salvage, which is integral to our obstetric patient blood management strategy, has resulted in an overall reduction in the number of women transfused allogeneic blood and the amount of blood transfused (figure one and table two). Compliance with our restrictive blood transfusion practice has been audited annually and presented locally and regionally to inform best practice [21-22]. As such the small increase in allogeneic blood use in 2014 was audited and presented locally to re-establish the principles of using blood wisely [21].

Our biggest group of obstetric patients requiring allogeneic transfusion are those who deliver vaginally, without the option for cell salvaged blood, with a smaller group of women who have a caesarean section requiring post-natal 'top-up' transfusions, despite a restrictive transfusion trigger. Not only has the percentage of women needing an allogeneic blood transfusion dropped to far lower than the suggested 5% standard [23], but as important, is the reduction of the number of units of blood transfused, which has reduced from a median of two units (IQR 2-3) in 2008 to less than two units after 2012 when the service was fully established (median 1, IQR 1-2) (table two).

Since 2016, the allogeneic transfusion rate has plateaued, mainly due to the women who bleed following a vaginal delivery who then require a blood transfusion. We have undertaken a feasibility study which shows it is possible to collect blood from vaginal blood loss, salvage it and potentially re-infuse. No patients were reinfused during this study, but the evidence supports the safe use of vaginal cell salvage in certain clinical situations [24]. The authors are supporting a further trial repeating this study [6].

Few women received tranexamic acid and it was not used routinely throughout the study period, however this has not been documented formally and use of tranexamic acid may have increased in 2017 following publication of the WOMAN study [25].

There are a number of perceived risks and barriers to implementing a routine cell salvage service in obstetrics, which include the potential for an increased risk of alloimmunisation secondary to the re-infusion of fetal red blood cells in the final processed cell salvaged blood. If cell salvage is routinely employed in obstetrics, formation of other clinically significant antibodies other than anti-D, that could impact on future pregnancies, must be considered. We have attempted to assess this risk by following up all women who have had a re-infusion of cell salvaged blood, three to four months post-delivery and checking their blood for antibody formation [26].

Of the 647 (55%) women who have received cell salvaged blood that have been tested for antibody formation, there have been two cases with a positive antibody screen. Both were anti-E. The first case was an abruption with an estimated blood loss of 600ml, receiving 237ml of cell salvaged blood and one-unit allogeneic transfusion (unit was E antigen negative). The second case was a twin vaginal delivery requiring urgent surgical intervention due to complications and bleeding, with the second baby being born by caesarean section an hour later than the first baby. 400ml of cell salvaged blood was reinfused avoiding the need for any allogeneic blood transfusion. The techniques used within our laboratory have a similar detection profile to enzyme methods for Rh antibodies, and as such these antibodies may be 'naturally occurring' (i.e. not as a result of red cell stimulus), but this cannot be proven with confidence.

Prior to the dataset reported in this paper, there was one other case with an antibody identified (anti-S) that was not detected antenatally. We were unable to establish whether the anti-S was produced due to contamination from the fetal red blood cells in the cell salvage re-infusion or before this, due to the multiple transplacental haemorrhages, antenatal bleeds and final abruption resulting in emergency delivery by caesarean section at 33 weeks gestation.

The SALVO randomised controlled trial [5] suggested that fetomaternal haemorrhage was higher in those women who had cell salvage than those who did not. However, data was only compared for cases where fetomaternal haemorrhage was greater or equal to 2 ml, and a RhD positive baby was born to a RhD negative mother using 30 cases: 21 in the cell salvage intervention group and nine in the control (non-cell salvage) group. Based on such low numbers we support the authors in stating that associating the alloimmunisation risk from these fetomaternal haemorrhage results should be treated with caution and no conclusion about alloimmunisation risk can be drawn from the results in SALVO.

Other recommendations suggest considering an increased dose of prophylactic anti-D required for RhD negative women post cell salvage reinfusions. From our data, over the last three years looking at all RhD negative women re-infused cell salvaged blood who delivered a RhD positive baby (N=60), we have not seen an increase in the dose of prophylactic anti-D being administered. Our hospital issues 1500 IU doses, sufficient to cover up to a 12ml bleed/contamination.

Other perceived barriers to implementing cell salvage in obstetrics include concerns about costs, manpower planning, and training. When cell salvage was first introduced to the maternity operating theatre it was only set up for elective caesarean sections. The ODPs, assisting the anaesthetist, gain their initial competencies in cell salvage in an elective setting before they progress to managing emergency cases. We have a dedicated cell salvage trainer who ensures all ODPs who are rostered to work in maternity theatre complete a competency-based training package which includes both theory and practical elements of training. Following publication of our data showing cell salvage was an important element of our blood conservation strategy [27], we have achieved a 24-hour, seven-day availability without any additional staffing, using the ODP who routinely assists the anaesthetist to manage all elements of the cell salvage operation.

At the time of the invitation to participate in the SALVO study, in 2012, our data had confirmed benefit of cell salvage in obstetric practice and we did not have equipoise to participate, already using it in 98% of cases and seeing an associated reduction in transfusion. Retrospective review of our data indicates many women who receive cell salvaged blood are women who have no pre-operative predictors for blood loss. By trying to predict, and only collect for those women at risk of bleeding, many women who bleed will be missed, and limiting the use of cell salvage to high-risk cases may result in reduced opportunities for training and maintenance of skills [28]. Data collected from 300 cases (2010-2013) showed the median estimated blood loss was 800ml (600-1000 [300-5500]). There was no correlation between the urgency of the caesarean section and re-infused volume, estimated blood loss, and Para gravida (correlation 0.253, -0.228, 0.014, respectively). This early data set confirmed the perception that those women at risk of bleeding cannot be predicted and built the case for using cell salvage routinely. There was weak correlation between estimated blood loss and length of stay (0.334), and moderate correlation between estimated blood loss and cell salvaged blood re-infused volume (0.535), which would be expected.

We have not performed a detailed cost analysis but feel recent estimates of cost [5] are considerably greater than the costs of the service we provide. Besides incurring no additional manpower costs, we have only ever used one suction device [5]. This saves on cost of a second suction and also results in more efficient blood collection, reducing waste. More UK hospitals are adopting the one suction approach with 58.1% of hospitals using one suction compared to 41.9% using two suction devices [5]. Further cost reductions are achieved when the ODPs do not open the cell salvage processing kit for all collection cases. They estimate blood loss and blood in the collection reservoir, only opening the processing kit when enough blood has been collected, which on processing is likely to fill a full bowl. Our data has shown that more than 60% of cases will be collection only and will not incur the additional expense of the processing kit which has been used in the calculation of costs in the SALVO study. Some of the collections when processed will be of insufficient volumes to fill the 125ml bowl in the automatic setting of the Haemonetics cell salvage machine. These processed collections are classed as partial first bowls and are therefore not always re-infused. Some of these women may require a transfusion one to two days later. The role of partial bowls has not been addressed in Obstetrics, but it is likely that re-infusing partial bowls will reduce the number of women having a top-up transfusion post-operatively.

The current recommendation to use a leucodepletion filter when re-infusing obstetric cell salvaged blood is supported with little to no evidence. Before 2000, this filter was not available, and many centres have abandoned their use or never used the filter, safely re-infusing cell salvaged blood without a problem. When blood loss is rapid, the flow rate through the filter may not be sufficient to give back large volumes of blood quickly. The use of a pressure cuff or syringing cell salvaged blood to increase rapidity of re-infusion is not advised due to the risk of air embolus and the unknown impact of pressure within the filter. Adverse events related to re-infusions given through a leucodepletion filter continue to be reported [5]. Reports to SHOT from 2010-2017 have identified 20 hypotensive episodes associated with using the leucodepletion filter [29] and in the most recently

published SHOT report (for the year 2017) [30] there were eight obstetric cell salvage cases reported, with three of these cases linked to the filter; two resulted in hypotension, and the third resulted in a reduced infusion rate resulting in time expired blood.

To maximise further use of cell salvage, and to reduce allogeneic rates, we are considering the use of partial first bowls for re-infusion. Although evidence for concern is limited and could just be theoretical, Haemonetics™ product literature now states FDA clearance to wash and reinfuse a partially-filled bowl to maximise red blood cell re-infusion [31], although information available to support this is limited. In earlier work, we have demonstrated that processing and washing of blood during cell salvage significantly reduced concentrations of α-fetoprotein to well within the normal range for the general population. Heparin is also eliminated, and while the washing process does not eliminate the presence of squames cells, their significance in the circulation remains unknown. [18, 24]. Currently quality control testing is performed monthly on an ad-hoc basis, however setting up a standardised quality control programme for cell salvage blood throughout the UK is something the authors support and are aware is undergoing consideration [personal communication Catherine Ralph, UKCSAG; 32]. We are investigating the quality of blood obtained from partial bowls compared to blood obtained from full bowls and allogeneic units (table three).

Another consideration to address when comparing allogeneic to autologous cell-salvaged blood transfusion is that 1 ml cell-salvaged blood is not similar in vivo to 1 ml of allogeneic blood. There is no data comparing cell-salvaged blood with allogeneic blood although our observational data suggest cell-salvaged blood behaves more like whole blood with women who receive large volumes of cell-salvaged blood maintaining normal coagulation and requiring little to no additional clotting factors. This can be seen in the low volumes of other blood component support needed, as seen in table two. Research into this in vivo observation is currently in progress at our hospital (IRAS: 225799; REC ref 17/NW/0586).

In concluding, we have shown it is possible to safely and economically employ intra-operative cell salvage routinely within an obstetric unit with no additional staffing costs so long as it is set up as collection only and used routinely for all cases. The risk of amniotic fluid embolus remains theoretical and fetal red cell contamination should not be barriers to implementing a cell salvage service. Despite the limited follow-up data of test for antibody formation, data so far suggests that receiving a re-infusion of cell salvaged blood does not put women at any more risk of antibody formation compared to women who receive an allogeneic transfusion. However, without more complete follow-up, rates may be deceptive, and as we have previously recommended, all Institutions using intra-operative cell salvage in obstetrics should adopt this method of following up women post re-infusion in order to collect alloimmunisation rates. Recording this data on a central database will allow sufficient data to be collected and to finally quantify the risk of alloimmunisation in obstetric cell salvage [26].

Based on the reported observational data collected over ten years, we recommend that leucodepletion filters are no longer used in obstetrics, although we would support further work to

evidence base this recommendation. The quality of blood processed from partial first bowls is no worse than that of full bowls (apart from haemoglobin/haematocrit), and that partial first bowls should be considered in obstetrics for re-infusion.

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Table 1 Intra-operative cell salvage collection and processing during caesarean section. Values are number (proportion).

Year	Caesarean sections	Collection	Processed	Proportion of caesarean sections with blood processed	Proportion of processed cases with reinfusion
2012	845	806 (95.4%)	228 (28.3)	27.0	53.1
2013	840	805 (95.8)	230 (28.6)	27.4	63.5
2014	869	853 (98.2)	258 (30.1)	29.7	55.0
2015	951	930 (97.8)	288 (31.0)	30.3	63.4
2016	826	814 (98.5)	264 (32.4)	32.0	66.7
2017	868	861 (99.1)	337 (39.1)	38.8	70.0

* Collection only ('stand-by') where lost blood is collected into the collection reservoir. Processing kits have not been opened yet.

Table 2 Royal Cornwall Hospital obstetric transfusion data from last ten years

	Total women delivered	Number of caesarean sections	Total number of women transfused allogeneic blood *	Total number of intra-partum women transfused allogeneic blood *	Percentage of intra-partum woman transfused	Number of caesarean section cases transfused	Total units transfused	Median number of units transfused (IQR [range])	Number of women who received cell salvaged blood	Number of women who received cell salvage and allogeneic blood	Number of women requiring fresh frozen plasma (FFP)	Number of women requiring cryoprecipitate	Number of women requiring platelets
2008	3278	585 +	79	58	1.3 *	15	259	2 (2-3 [1-20])	20	2	9	1	6
2009	4354	764	64	43	1.0	22	192	2 (2-3 [1-16])	25	2	6	3	5
2010	4492	801	59	44	1.0	20	155	2 (1-2 [1-13])	34	8	7	3	4
2011	4769	876	58	40	0.8	5	167	2 (1-3 [1-27])	87	5	7	2	2
2012	4628	845	48	35	0.8	12	100	2 (1-3 [1-5])	121	6	9	0	0
2013	4612	840	39	31	0.7	8	75	2 (1-2 [1-6])	146	4	5	1	0
2014	4388	869	54	39	0.9	17	112	2 (1-2 [1-10])	142	10	8	2	2
2015	4316	951	21	9	0.2	1	31	1 (1-2 [1-3])	183	1	2	0	0
2016	4338	826	27	18	0.4	3	57	2 (1-2 [1-11])	176	3	2	1	2
2017	4089	868	24	16	0.4	7	39	1 (1-2 [1-4])	236	3	2	1	1

*Total number of women transfused allogeneic blood include ante-natal, peri-natal and post-natal women, whereas Intrapartum women are those who were transfused on the Delivery Suite or immediately post theatre.

+ Due to change in computer system, not all data is available from 2008. Data reported is from nine months (April-December)

Table 3 Quality of blood obtained from cell salvage blood full bowls, compared with partial bowls and allogeneic red cells. Values are median (IQR [range])

	Full bowls (N=77)	**Partial bowls (N=40)**	**Allogeneic * (N=60)**
Cell salvage volume (ml)	152 (127-229 [106-732])	122 (118-126 [95-132])	X
Haemoglobin ($g.l^{-1}$)	156 (140-171 [110-215])	101 (78-115 [24-135])	197 (183-205 [163-219])
Haematocrit ($l.l^{-1}$)	0.461 (0.412-0.495 [0.348-0.662])	0.295 (0.241-0.331 [0.075-0.423])	0.659 (0.626-0.690 [0.513-0.728])
Albumin + ($mg.l^{-1}$)	6.4 (4.2-16.6 [0.1-941.0])	7.5 (4.4-11.0 [1.3-113.4])	>500
Heparin ($iu.ml^{-1}$)	0.00 (0.00-0.00 [0.00-0.55])	0.00 (0.00–0.00 [0.00–0.08])	X
Potassium ($mmol.l^{-1}$)	1.6 (1.2-2.1 [0.6-10.2])	0.9 (0.6-1.2 [0.4-2.9])	38.5 (30.0-49.0 [17.0-71.0]) ‡
Lactate dehydrogenase ($iu.l^{-1}$)	243 (171-336 [67-1709])	140 (111-200 [42-763])	127 (79-196 [38-596])
Plasma free haemoglobin § ($g.l^{-1}$)	1.20 (0.22-2.50 [0.02-13.50])	0.26 (0.03-0.90 [0.00-5.60])	0.90 (0.58-1.80 [0.20-4.80])

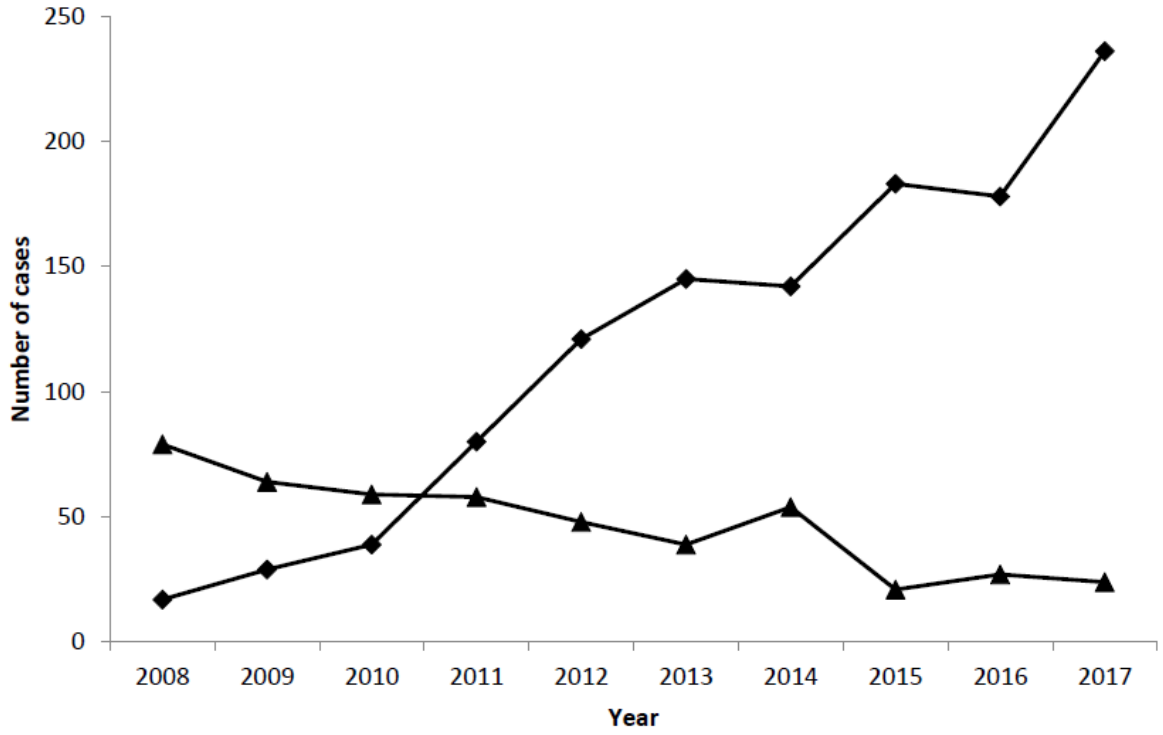
* Allogeneic units were leucodepleted and stored in SAGM. Samples were either taken from wasted or time expired units that were still within the cold chain criteria, or from the unit giving set prior to connecting to a patient's cannula. Again, no needles were used. Blood was collected into EDTA. Average age of the blood was 21 days.

+ Albumin results obtained from Microalbumin test.

‡ Data only available from 36 samples

§ Plasma free haemoglobin levels tested on a HemoCue Plasma/Low Haemoglobin System (HemoCue Ltd, Derbyshire, UK).

Figure 1 Number of intra-partum woman transfused cell salvaged blood on the Delivery Suite or immediately post theatre (square) and allogeneic blood (triangle)



FORM UPR16

Research Ethics Review Checklist

Please include this completed form as an appendix to your thesis (see the Research Degrees Operational Handbook for more information

UNIVERSITY OF PORTSMOUTH

Postgraduate Research Student (PGRS) Information			Student ID:	899202
PGRS Name:	Mr Ian Sullivan			
Department:	Faculty of Science	First Supervisor:	Professor Graham Mills	
Start Date: (or progression date for Prof Doc students)	February 2018			
Study Mode and Route:	Part-time ☐ Full-time ☒	MPhil ☐ PhD ☒	MD ☐ Professional Doctorate ☐	

Title of Thesis:	Implementation and on-going improvement of an obstetric intra-operative cell salvage service
Thesis Word Count: (excluding ancillary data)	10,873

If you are unsure about any of the following, please contact the local representative on your Faculty Ethics Committee for advice. Please note that it is your responsibility to follow the University's Ethics Policy and any relevant University, academic or professional guidelines in the conduct of your study

Although the Ethics Committee may have given your study a favourable opinion, the final responsibility for the ethical conduct of this work lies with the researcher(s).

UKRIO Finished Research Checklist:
(If you would like to know more about the checklist, please see your Faculty or Departmental Ethics Committee rep or see the online version of the full checklist at: http://www.ukrio.org/what-we-do/code-of-practice-for-research/)

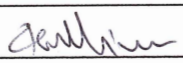
a) Have all of your research and findings been reported accurately, honestly and within a reasonable time frame?	YES ☒ NO ☐
b) Have all contributions to knowledge been acknowledged?	YES ☒ NO ☐
c) Have you complied with all agreements relating to intellectual property, publication and authorship?	YES ☒ NO ☐
d) Has your research data been retained in a secure and accessible form and will it remain so for the required duration?	YES ☒ NO ☐
e) Does your research comply with all legal, ethical, and contractual requirements?	YES ☒ NO ☐

Candidate Statement:

I have considered the ethical dimensions of the above named research project, and have successfully obtained the necessary ethical approval(s)

Ethical review number(s) from Faculty Ethics Committee (or from NRES/SCREC):	47.11.05 07/H0203/257 12/SW/0316

If you have *not* submitted your work for ethical review, and/or you have answered 'No' to one or more of questions a) to e), please explain below why this is so:

Signed *(PGRS)*:		**Date:** 14/01/2019